Aplicación de la Citometría de Flujo en la Práctica Clínica

ABA- Grupo Rioplatense de Citometría de Flujo

Estudio de inmunodeficiencias Primarias

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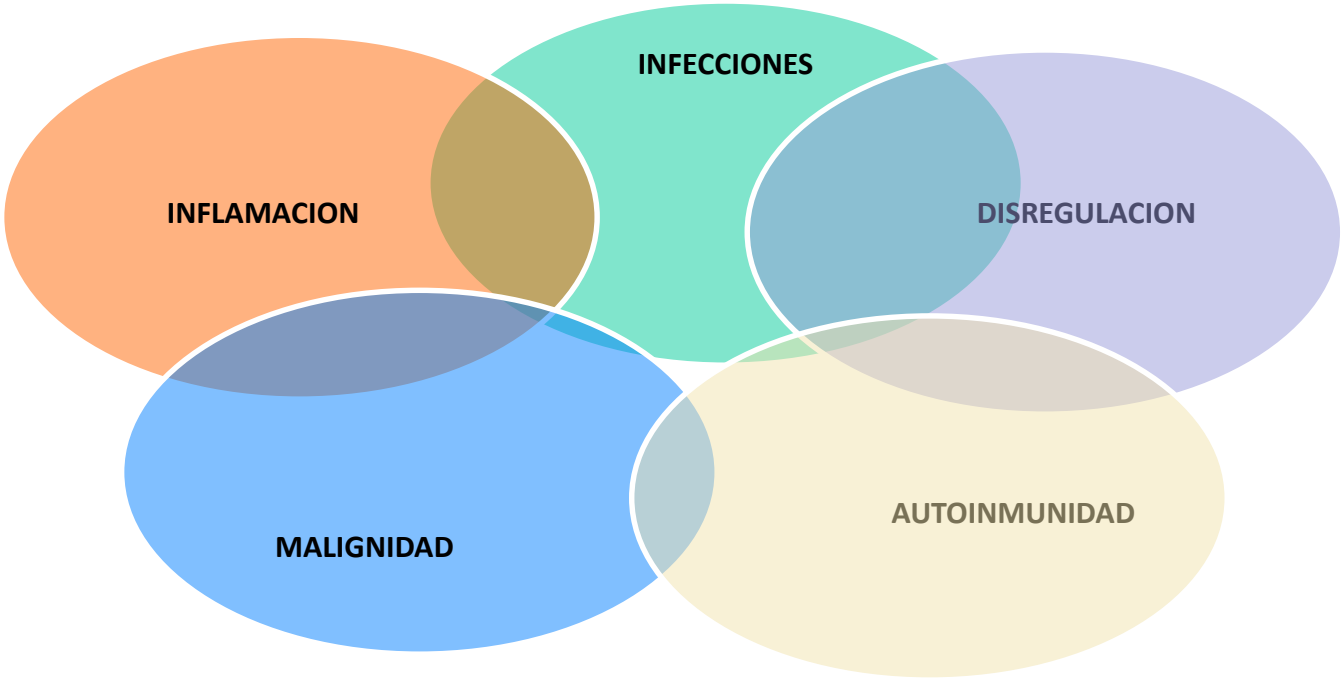
INMUNODEFICIENCIAS PRIMARIAS

Grupo heterogéneo y poco frecuente de enfermedades genéticas caracterizadas por alteraciones cuantitativas y/o cualitativas que comprometen en forma individual o combinada distintos componentes del sistema inmune



DEFECTOS DE INMUNIDAD INNATA

- **Defecto de neutrófilos**

Inicio: infancia

Presentaciones: onfalitis, abscesos, estomatitis, adenitis.

- **Defecto de macrófagos**

Inicio: infancia

Presentaciones: infección por micobacterias, infección intracelular.

- **Defecto de receptores tipo Toll (TLR)**

Inicio: infancia

Presentación: S. neumoniae, S. aureus, encefalitis por VHS

- **Defecto del complemento**

Inicio: cualquier edad

Presentación: trastornos reumatológicos (lupus, glomerulonefritis)

Infecciones: S. neumoniae, Neisseria sp.

DEFECTOS DE INMUNIDAD ADAPTATIVA

- Defectos de las células B

Inicio: después de los 6 meses de edad

Presentaciones: infecciones recurrentes sinopulmonares y / o crónicas gastrointestinales

Infecciones: bacterias capsuladas

- Defectos de células T o células T y B combinadas

Inicio: generalmente antes de los 6 meses de edad (puede ocurrir en adultos)

Presentaciones: falla de crecimiento, diarrea crónica

Infecciones: virus, hongos, patógenos oportunistas (pneumocistis, micobacterias)

INMUNODEFICIENCIAS CON DISREGULACION

- **Defecto en el desarrollo y tolerancia T**

APECED / IPEX (FOXP3)

IPEX like : STAT5b,CD25,hCTLA4,LRBA,STAT1GOF, STAT3GOF

- **Defecto en la resolución de la inflamación**

ALPS: Adenopatía crónica ,esplenomegalia, defectuosa apoptosis linfocitaria FAS, FASL

ALPS like: STAT3GOF, LRBA, PI3K

- **Linfohistiocitosis hemofagocítica (HLH)**

Con hipopigmentación : Chediak Higasi , Grisseli

Sin hipopigmentación: Perforinas Defectos en la degranulación

Suceptibilidad a VEB: XLP1/2 CD27 CD70

INMUNODEFICIENCIAS PRIMARIAS

HISTORIA CLÍNICA

Tipo, gravedad y frecuencia de las infecciones.
Tasa de crecimiento y desarrollo

HISTORIA FAMILIAR

Otros miembros de la familia afectados
Historia de abortos de repetición

PRESENTACION CLINICA

Sospecha de IDCS

- < 1 año
- Severas infecciones recurrentes (patógenos inusuales)
- Falla de crecimiento

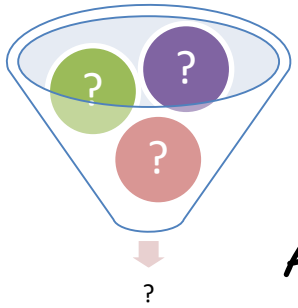
ID

- Recurrentes y/o inusuales infecciones
- Recurrentes infecciones por un mismo patógenos.
- Autoimmunidad, inflamacion y/o linfoproliferación

Sospecha IDH

- Recurrentes infecciones respiratorias: otitis media, sinusitis Bronquitis y neumonias

SOSPECHA DIAGNOSTICA



ALGORITMO DIAGNOSTICO

SOSPECHA DE DEFICIENCIA DE ANTICUERPOS

Cómo estudiarla ?

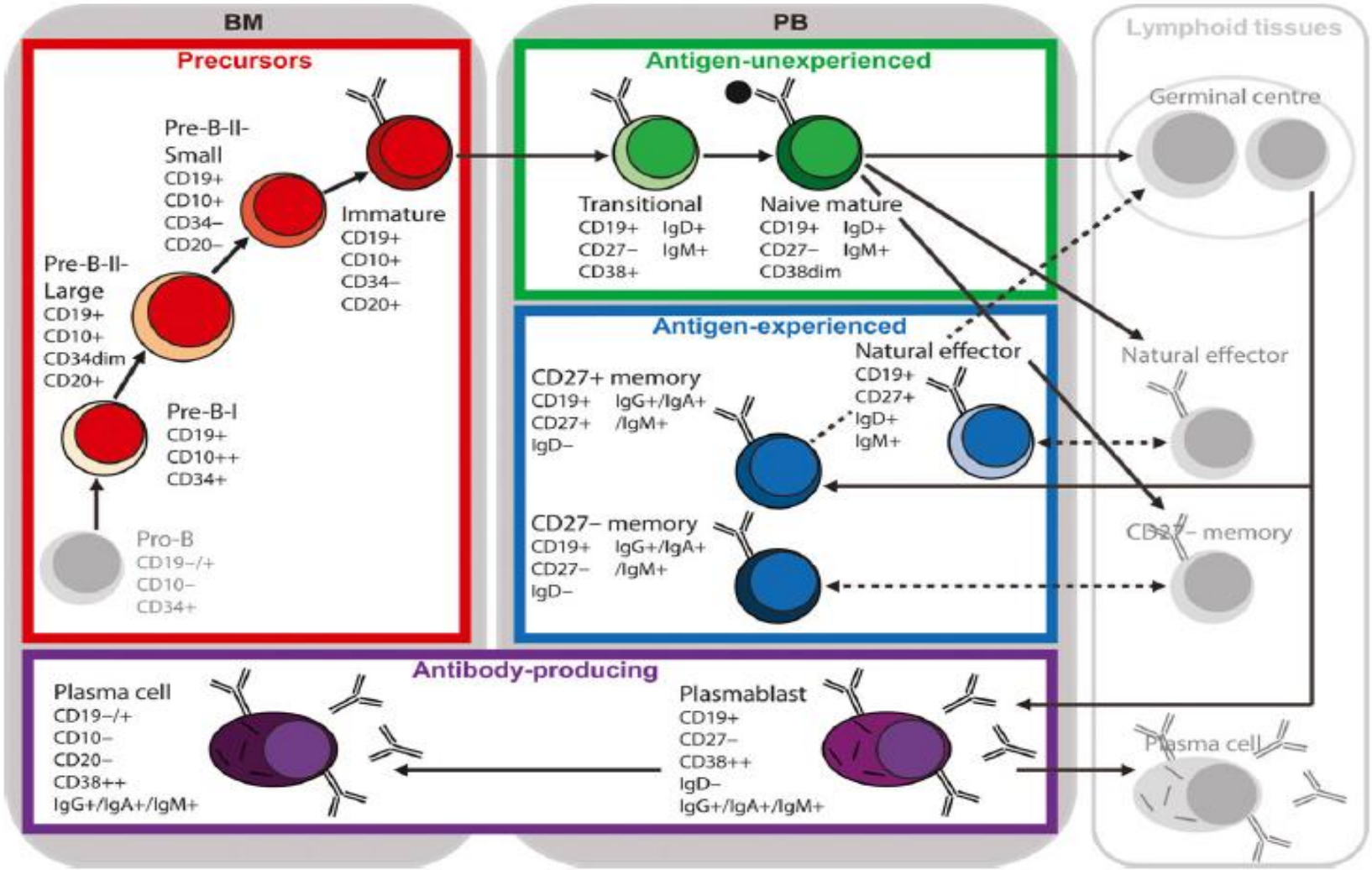
1. Dosajes séricos de Igs: IgG, IgM, IgA, IgE
2. Funcionalidad de Anticuerpos
3. Cuantificación de LB: CD19/CD20
4. Cuantificación de subpoblaciones naive /memoria B

FUNCIONALIDAD DE ANTICUERPOS

- **Naturales: isohemaglutininas IgM**
- **Post-infecciosos: ASTO, virales**
- **Post-vaccinales:**
  1. **Antígenos proteicos: HBV, Rubeola,TT, TD.**
  2. **Antígenos polisacáridos : neumococo**

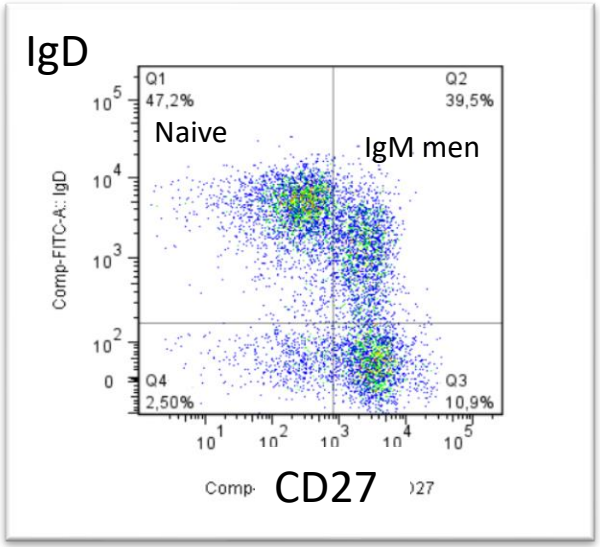
Dosajes de subclases de IgG : IgG1, IgG2, IgG3, IgG4

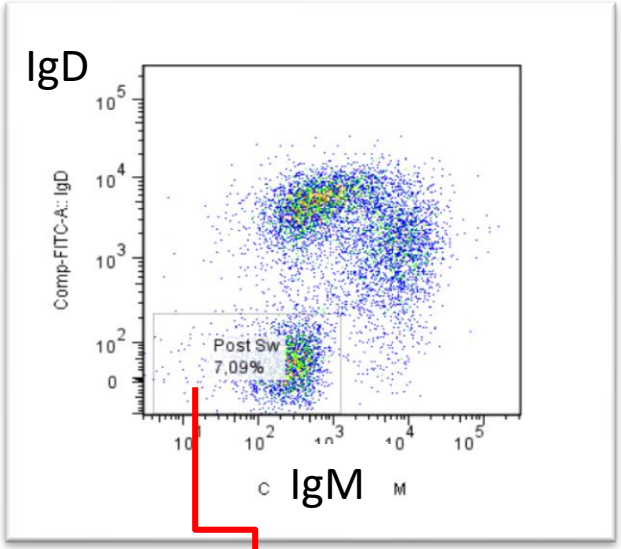
ONTOGENIA B - SUBPOBLACIONES DE LINFOCITOS B

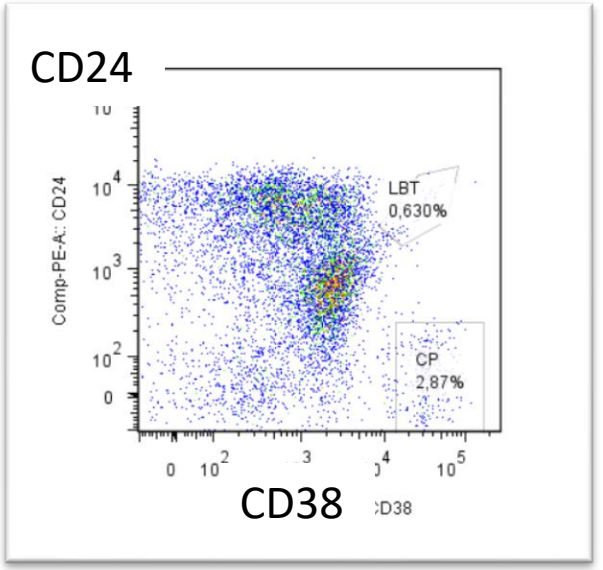


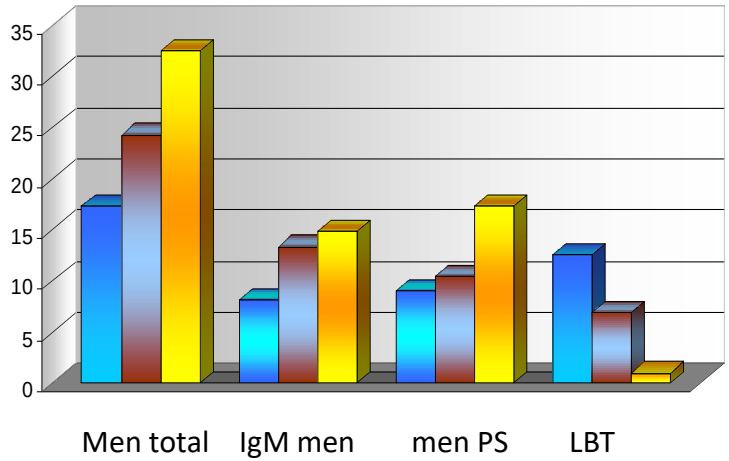
Prisca Theunissen et al
British Journal of Hematology January 2017

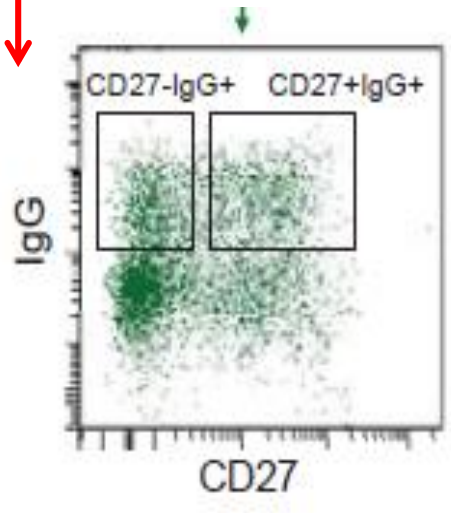
SUBPOBLACIONES DE LINFOCITOS B

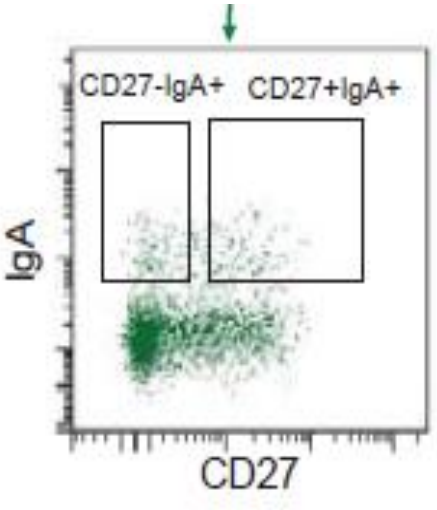












SOSPECHA DE DEFICIENCIA DE ANTICUERPOS

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CASO #1

10 a varón con historia de infecciones respiratorias recurrentes

Interconsulta por otitis supurada y neumonía

Antecedentes familiares : 3er hijo de padres no consanguineos sin historia familiar relevante

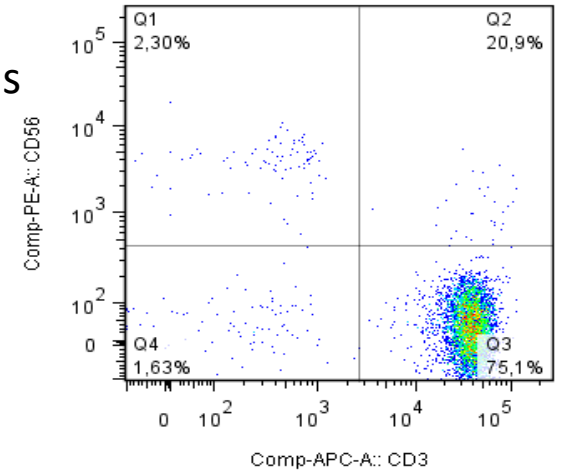
Laboratorio

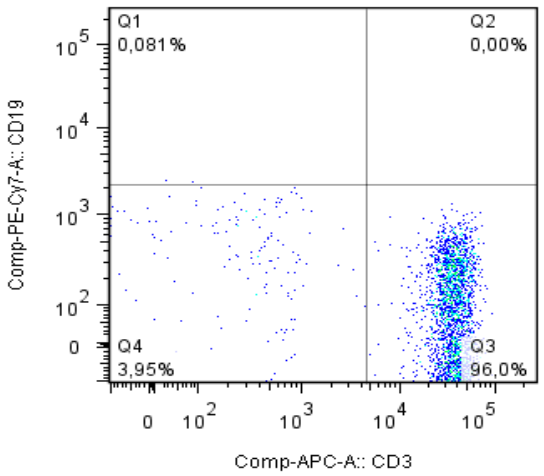
- IgG, IgA e IgM < 2DS
- Respuesta a Ags proteicos alterada

Subpoblaciones linfocitarias

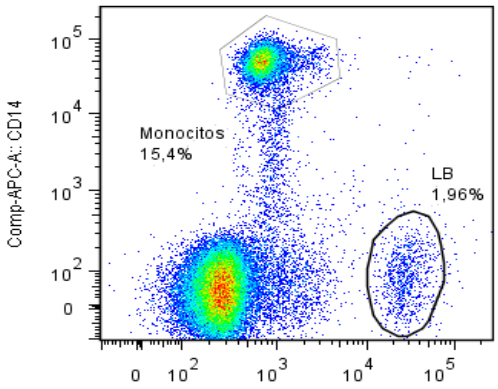
Con LB : < 1%

Molecular: **BTK(p.P565L)**

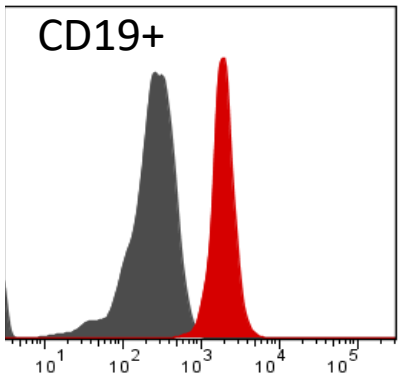




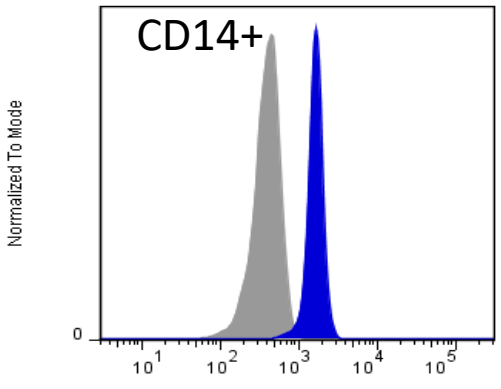
Expresion BTK

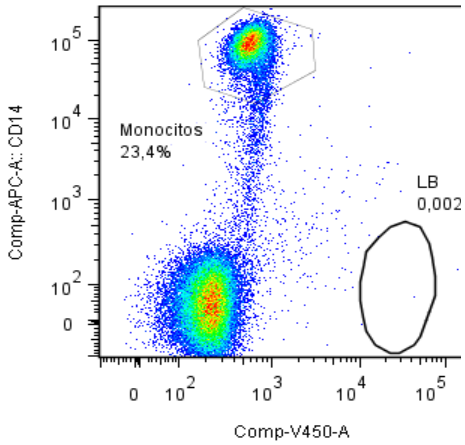


LB control

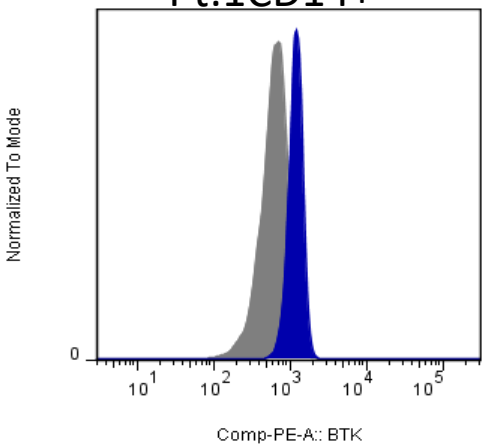


Monocitos control

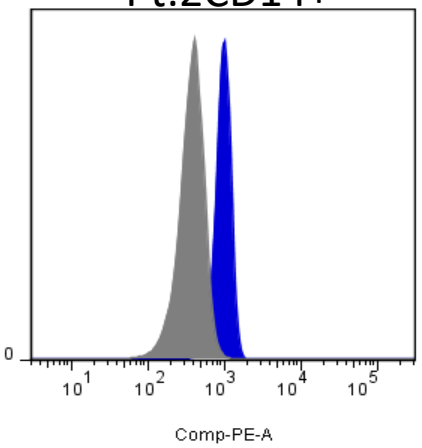




Pt:1CD14+



Pt:2CD14+



CASO #2

- Mujer 8a con antecedentes de **BOR** desde los 2 años de vida
- **Neumonías** (3,6 y 7 años) **recurrentes. Otitis supuradas** desde los 6 años. Varicela a los 4 años sin complicaciones. **Diarrea crónica**
- Antecedentes Familiares Madre: Deficiencia selectiva de IgA
- Laboratorio

Dosajes
• IgG: 310 mg/dl (900-1370)
• IgA: 10 mg/dl (80-220)
• IgM: 80 mg/dl (80-173)
• IgE: 15 UI/ml

Funcionalidad de Acs
• IgG sarampión: (-)
• IgG rubéola: (-)
• IgG varicela: (-)
• Isohemaglutininas: Grupo O Rh +
• Anti-A y anti-B: 1/2
• Ac neumoccócicos: 4 mg/l

- Poblaciones Linfocitarias CD3 CD4 CD8 CD20 CD19 NK normales
- Subpoblaciones de linfocitos B:

LB naive: 96%,

LB memoria totales: 2%,

LB memoria post cambio de isotipo PS: 1%

LBTransicional /inmaduros: 21%

CD21low: 6% VR: < 9%

Plasmoblastos: 0%

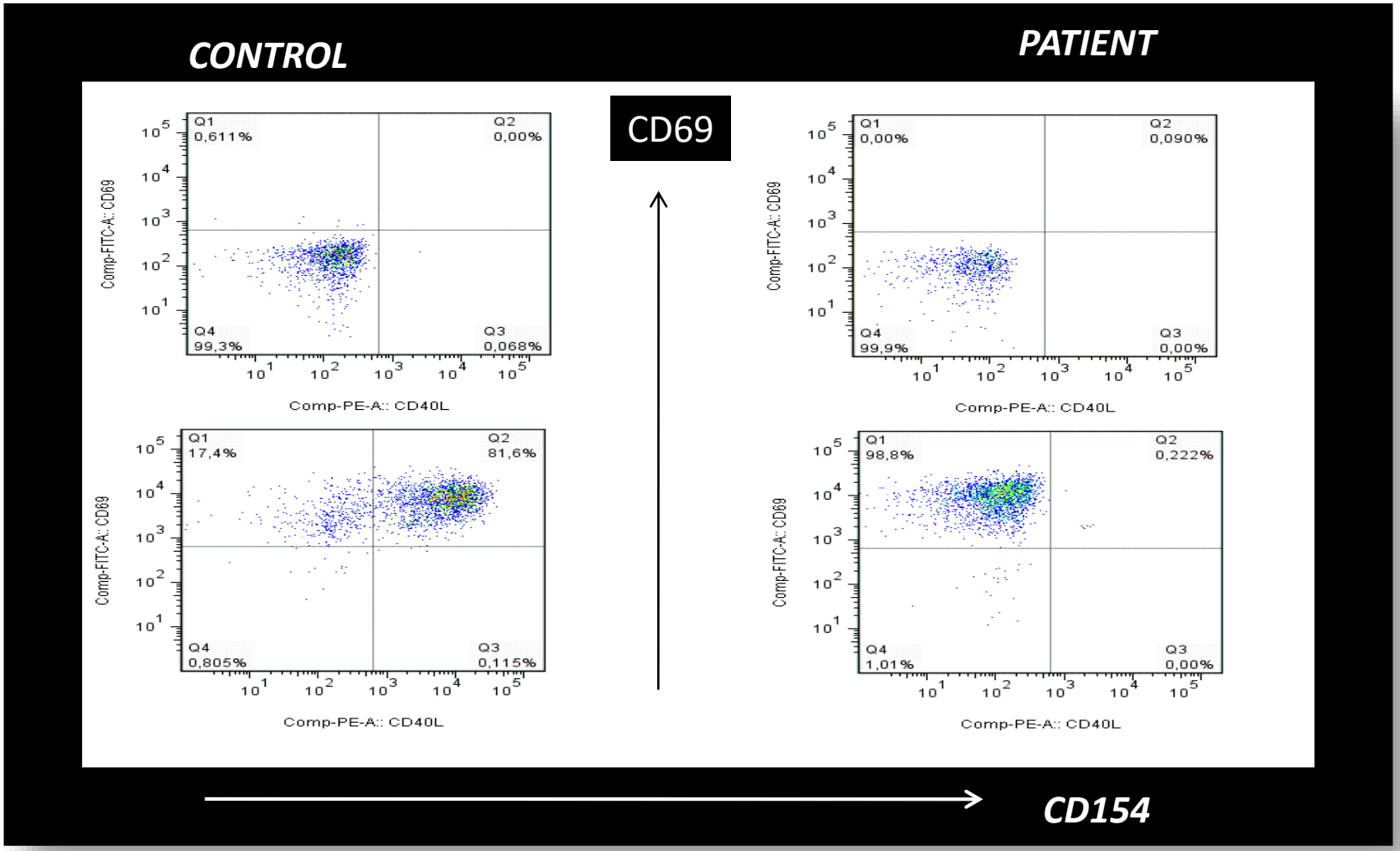
CASO #3

- Varón de 9 meses que presenta Pseudomona spp. y neutropenia. Comenzó el tratamiento antibiótico y factor estimulante de granulocitos. Neutropenia persistente.
- A los 2 años presentó adenomegalia cervical, con PCR (+) paraTBC

Laboratorio

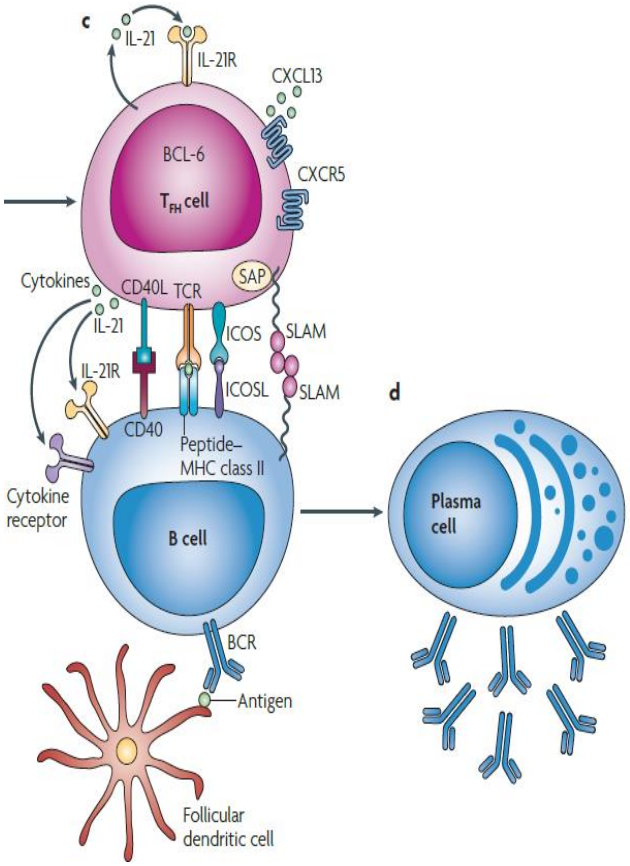
1. VIH negativo.
2. Dosajes IgG:28mg/dl; IgA: 4mg/dl; IgM: 77mg/dl
3. *Isohemaglutininas alteradas*
4. Subpoblaciones linfocitarias y respuesta proliferativa normal
5. Test DHR normal

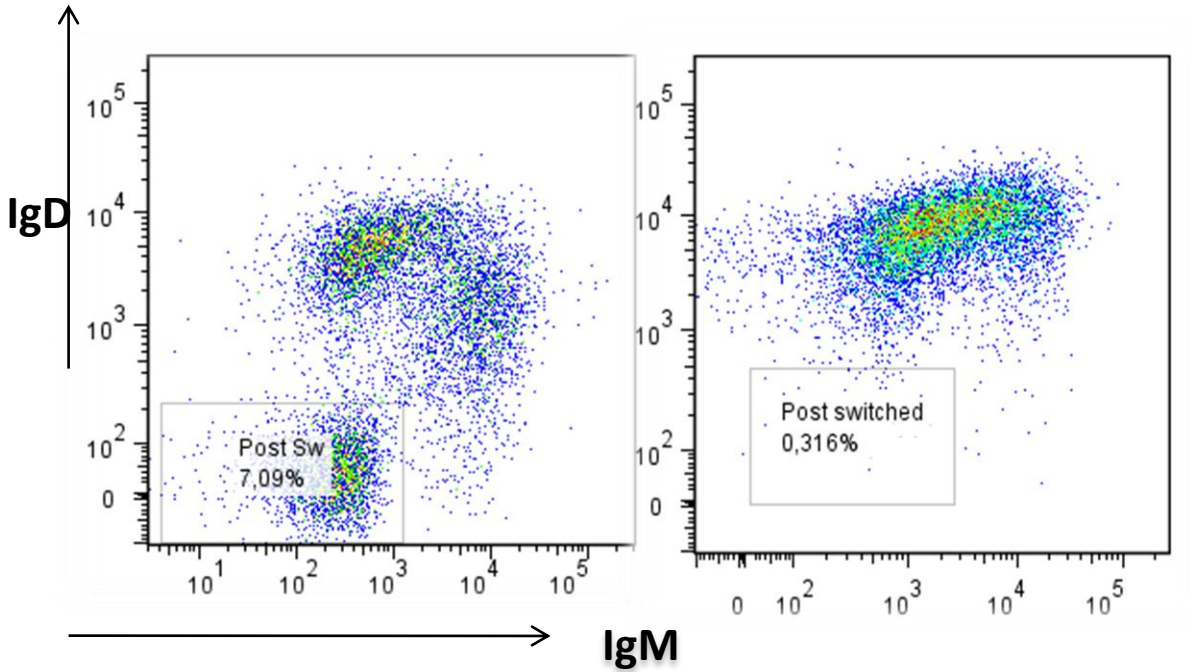
CD40L expresión en T CD4+ (I/PMA)



CD40L (p.Q174X)

Thf and Germinal Center





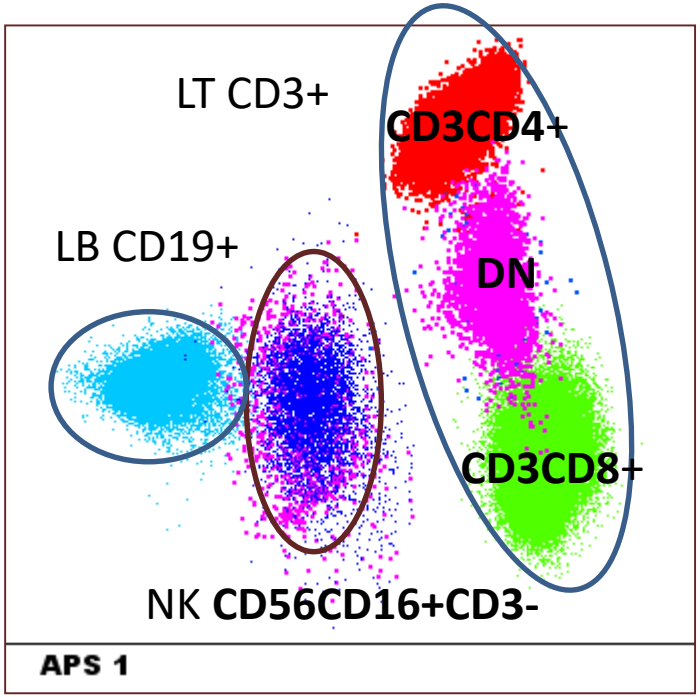
SOSPECHA DE DEFICIENCIA T o COMBINADA

Como estudiarla ?

1. • Hemograma completo
2. • Cuantificación de LT (output tímico vs sobrevida), LB,NK
3. • Cuantificación de subpoblaciones naive /memoria T (diferenciación)
4. • Respuesta proliferativa frente a diferentes estímulos (función)

2

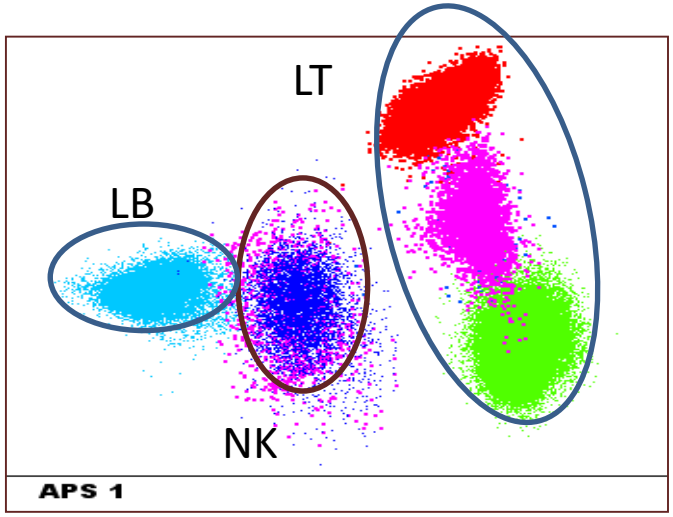
• Cuantificación de LT, LB,NK



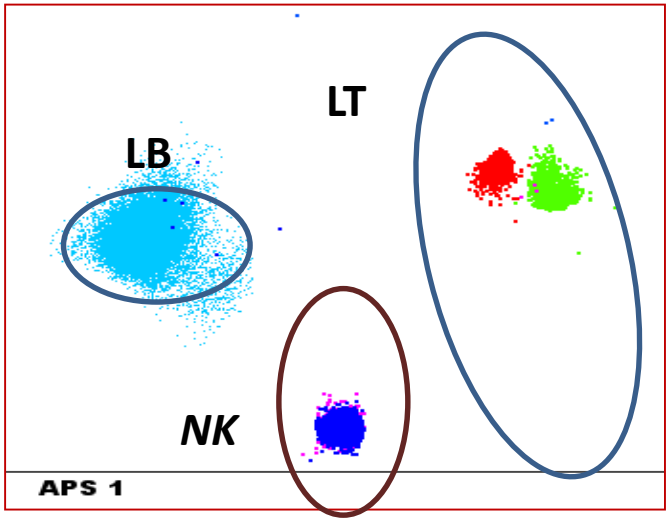
NORMAL

• Cuantificación de LT, LB,NK

ENFERMEDAD	FENOTIPO	DEFECTO MOLECULAR
X-SCID	T- B+ NK-	Cadena γ común R-ILs
Jak 3	T- B+ NK-	Jak 3
IL7Ra	T- B+ NK+	Cadena alfa IL7R
Cadenas de CD3	T- B+ NK+	Cadena δ ε ξ de CD3
SCID	T- B+ NK+	Coronina 1A
SCID por defectos recomb.	T- B- NK+	Rag 1, Rag 2,Artemis DNA-PKcs
Def. reparación DNA	T+/- B+/- NK+	Lig4 Cernunnos
Def. de ADA	T- B- NK-	ADA
Disg. reticular	T-B-NK- Neutropenia	AK2 (adenilato kinasa)

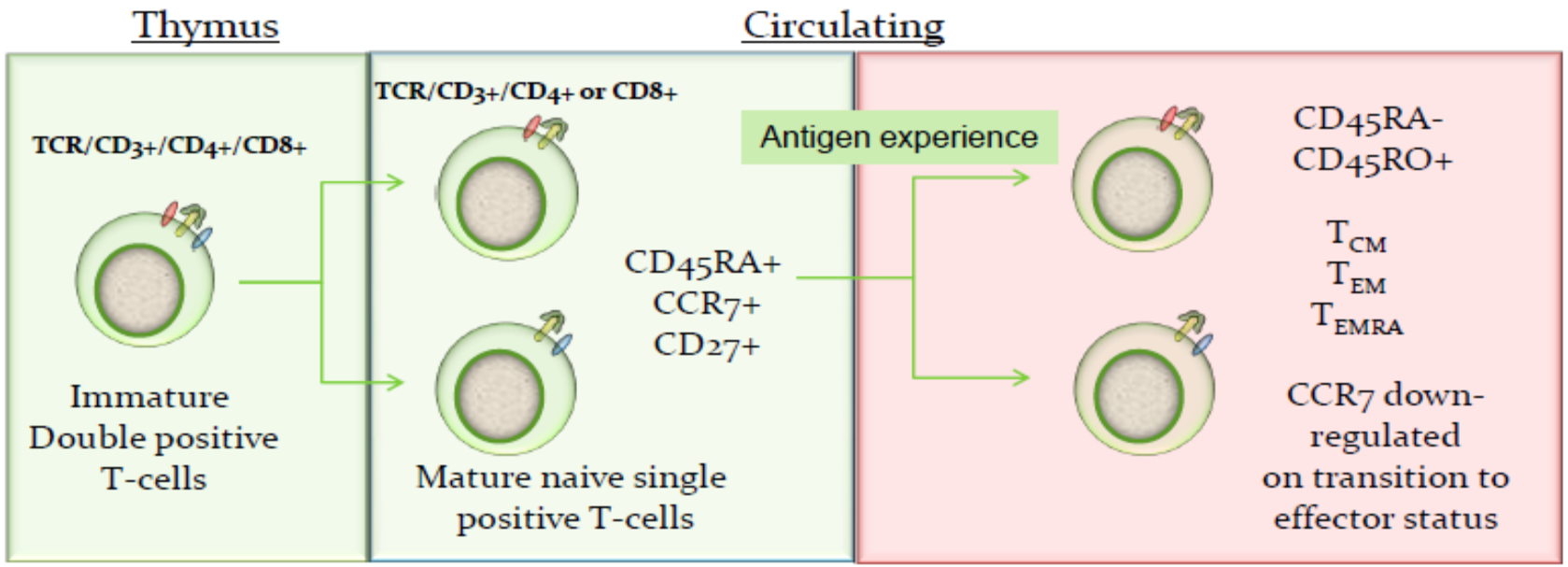


NORMAL



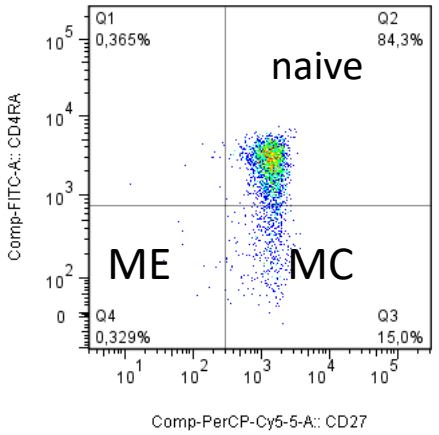
T-B+NK-

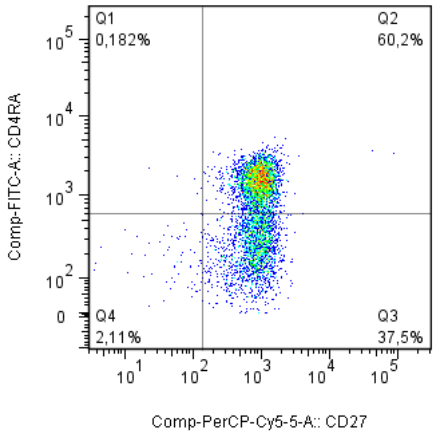
- Cuantificación de subpoblaciones naive /memoria T

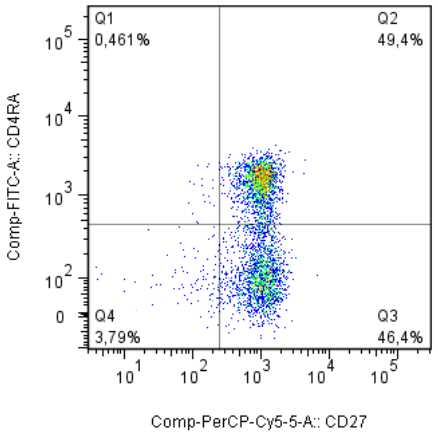


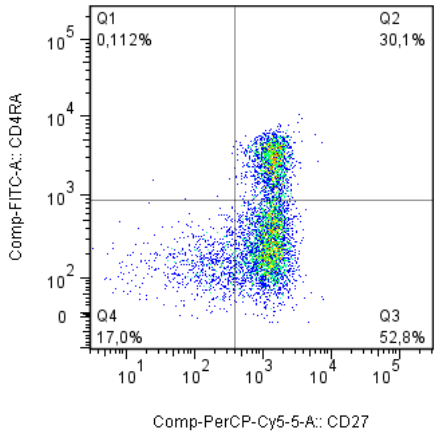
3

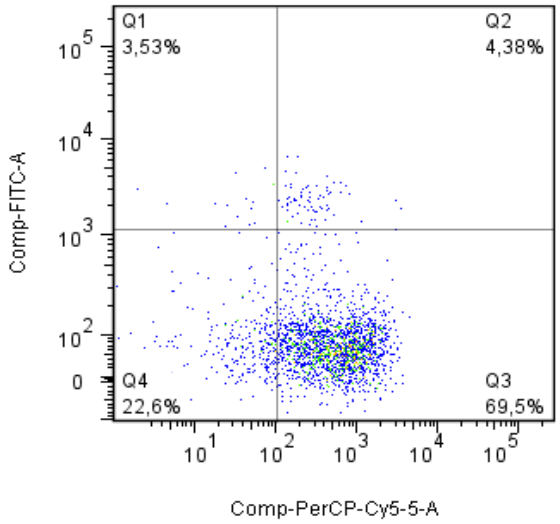
- Cuantificación de subpoblaciones naive /memoria T







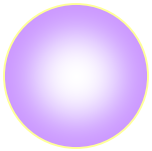




IDCS

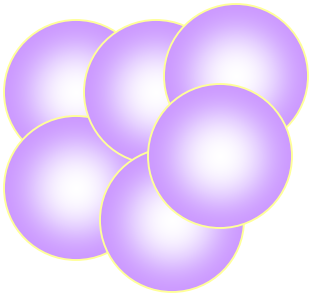
Funcionalidad Celular

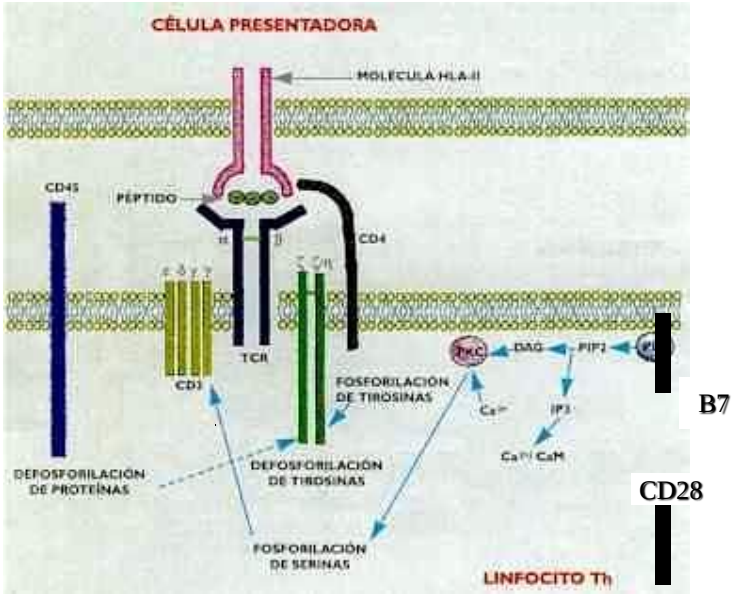
LINFOCITOS DEL PACIENTE



+ ESTIMULO

72hs a 96hs





Evaluar proliferación

Estímulos

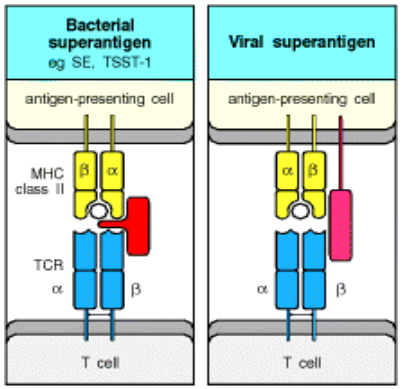
Lectinas PHA Con A PWM

•Desencadenan una rta. policlonal u oligoclonal, no es necesaria la exposición previa al antígeno, ya que no se produce reconocimiento específico del compuesto mitogénico.

• Se observa una rta. global, de los clones presentes en el individuo en estudio.

• No siempre se requiere de células presentadoras de antígeno.

SEB



Son moléculas que estimulan a un subconjunto de LT

por medio de la unión a las moléculas MHC de clase II

y al dominio Vβ del receptor T, estimulando la act. De LT

que expresan un determinado segmento Vβ.

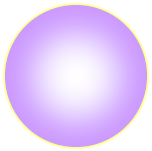
Acs. CD3 CD28 Ionomicina PMA

Ags candidina PPD TT

Desencadenan una rta. de memoria, por lo debe presentar contacto previo con el antígeno a ensayar. Se observa exclusivamente la respuesta de los clones que reconozcan al Ag.Se requiere de células presentadoras de antígeno

Proliferación Celular T

I- Activación Linfocitaria in vitro



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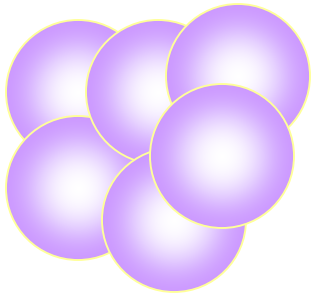


Se marcan las CMN con una sonda fluorescentes antes de activar (CFSE)

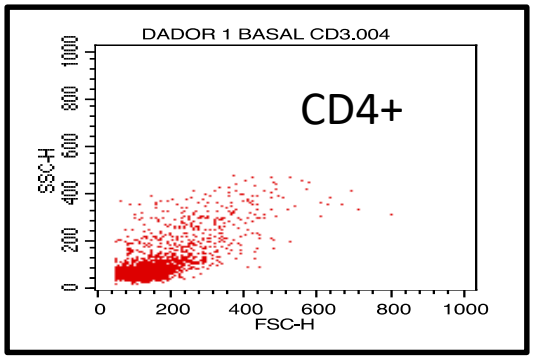
Al final del cultivo H-Timidina o EdU

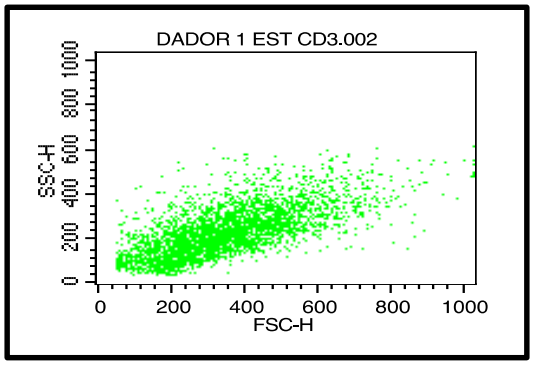
II - Cuantificación de la Rta

Conteo radioactividad (cpm)
Incorporación de EdU (CF) % +

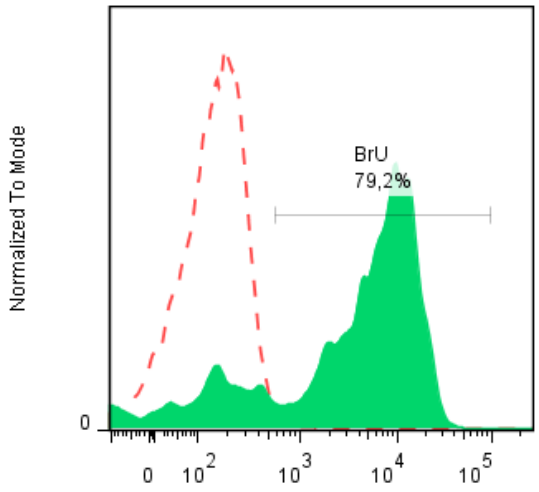
Disminución de fluorescencia de la sonda (CF)

Proliferación Celular : CITOMETRIA DE FLUJO: EdU

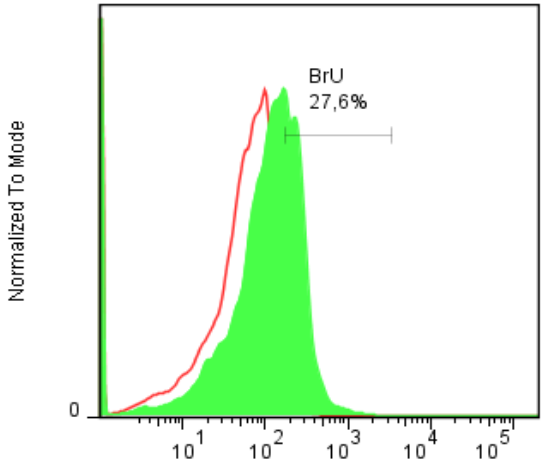




Control



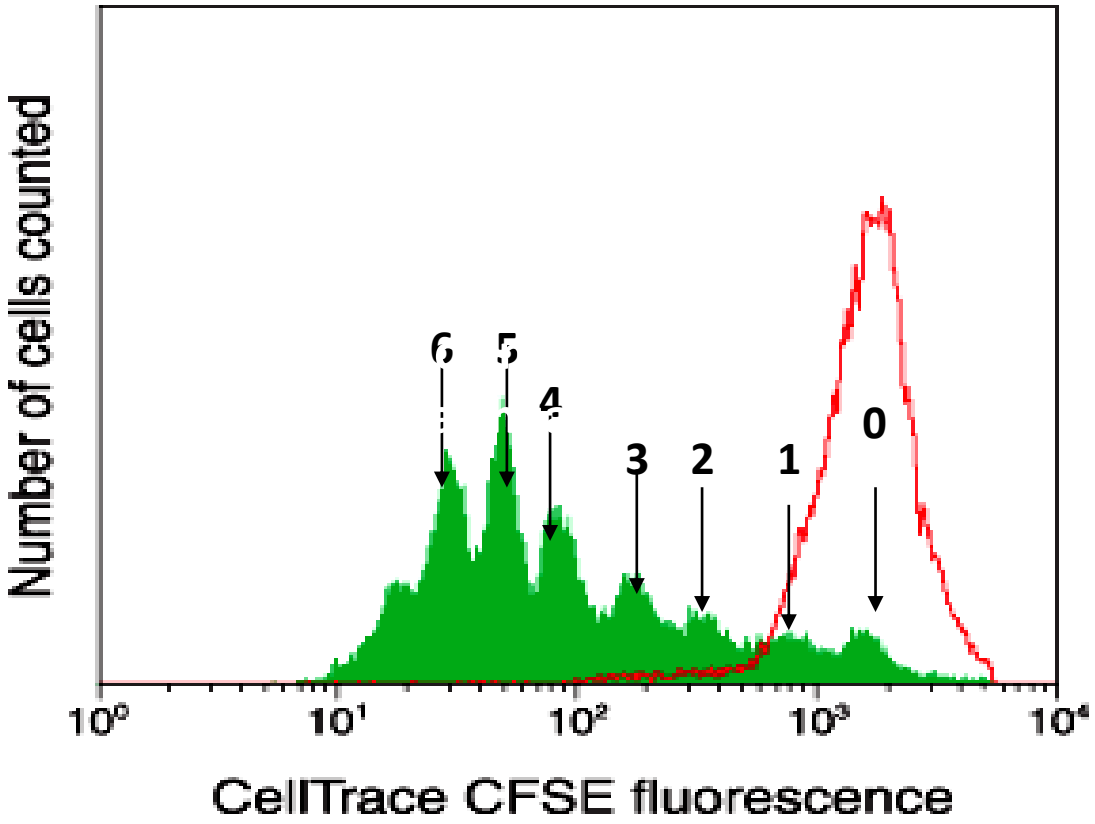
Paciente



- ✓Correlaciona con el ensayo de ^{3}H Timidina si la selección de la población se realiza sobre el total de linfocitos CD45+
- ✓No necesita del uso de material radioactivo
- ✓Permite evaluar proliferación de distintas subpoblaciones linfocitarias
- ✓Necesita de un citómetro de Flujo

Proliferación de Linfocitos

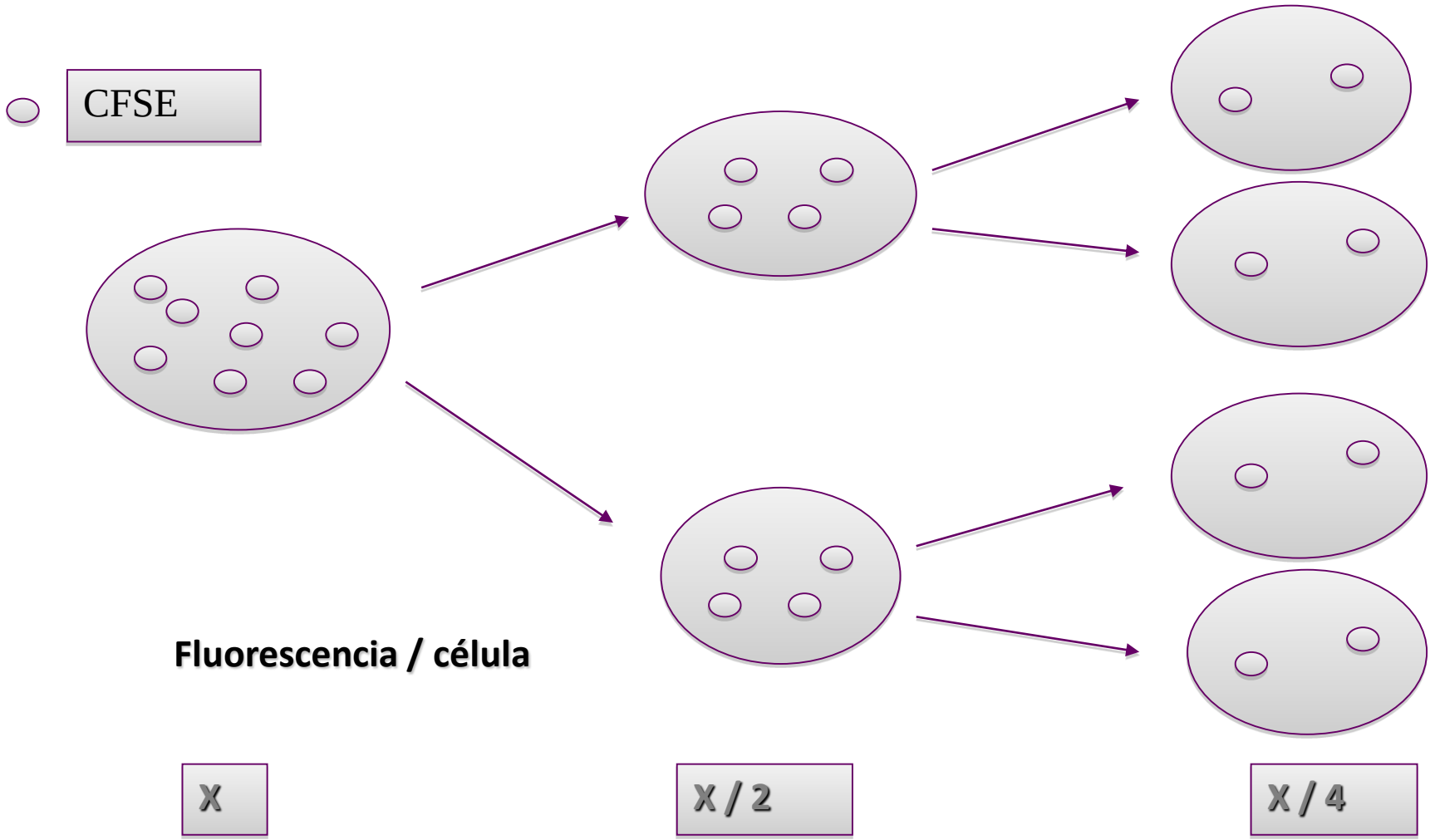
Ensayo de CFSE Carboxifluorescein diacetato succinimidil ester)



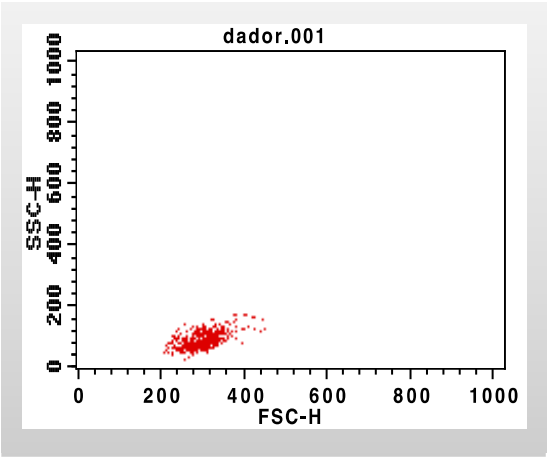
Sonda fluorescente que se une a proteínas intracelulares y que se reparte por igual entre las células hijas

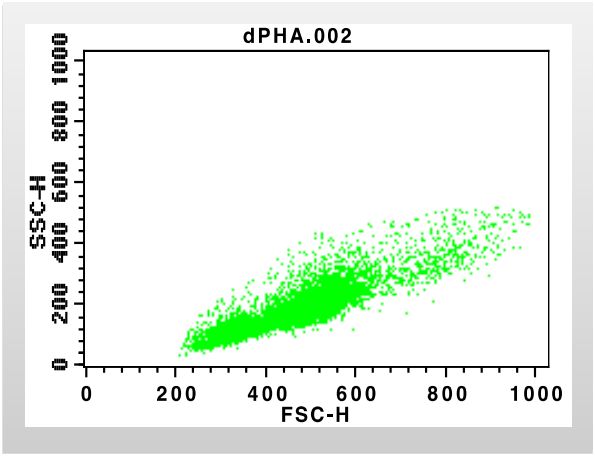
Evaluación de la respuesta inmune celular:

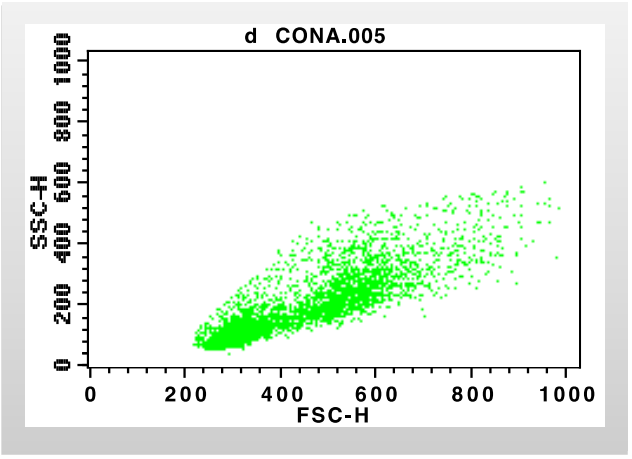
Historial de divisiones: CFSE

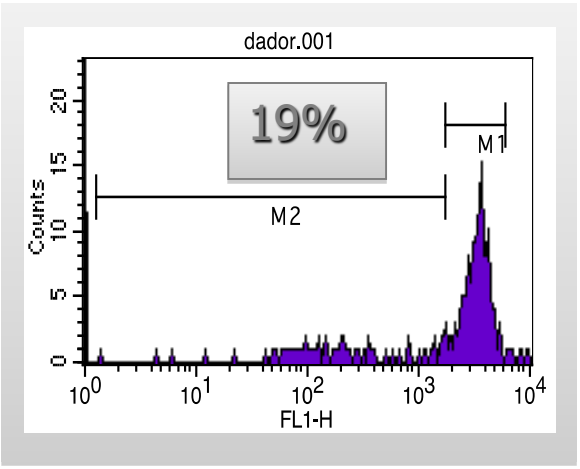


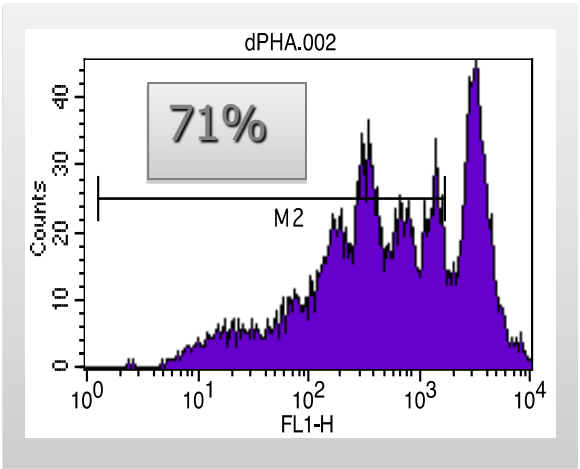
Marcación CFSE

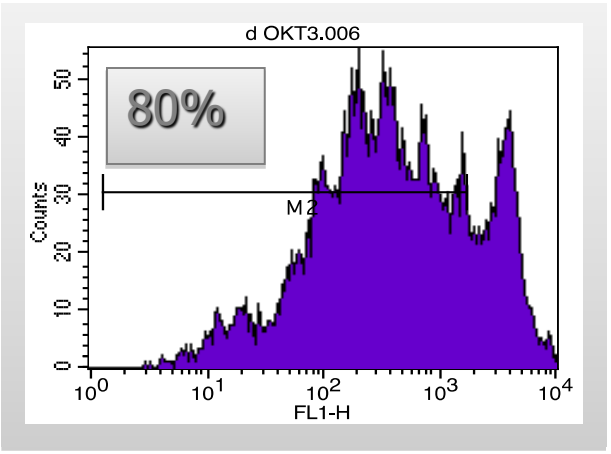












	CD4 (%)		CD8 (%)	
	P_{5-95}	P_{25-75}	P_{5-95}	P_{25-75}
PHA	45-96	65-88	55-98	68-91
OKT3	35-92	51-75	43-93	69-85
SEB	47-89	66-84	34-89	34-89

Desordenes de neutrófilos

Clínica: infecciones recurrentes respiratorias y de piel por hongos y bacterias
Recuento de PMN: disminuido : arrestos madurativos en la diferenciación
alto en defectos de la movilidad LAD

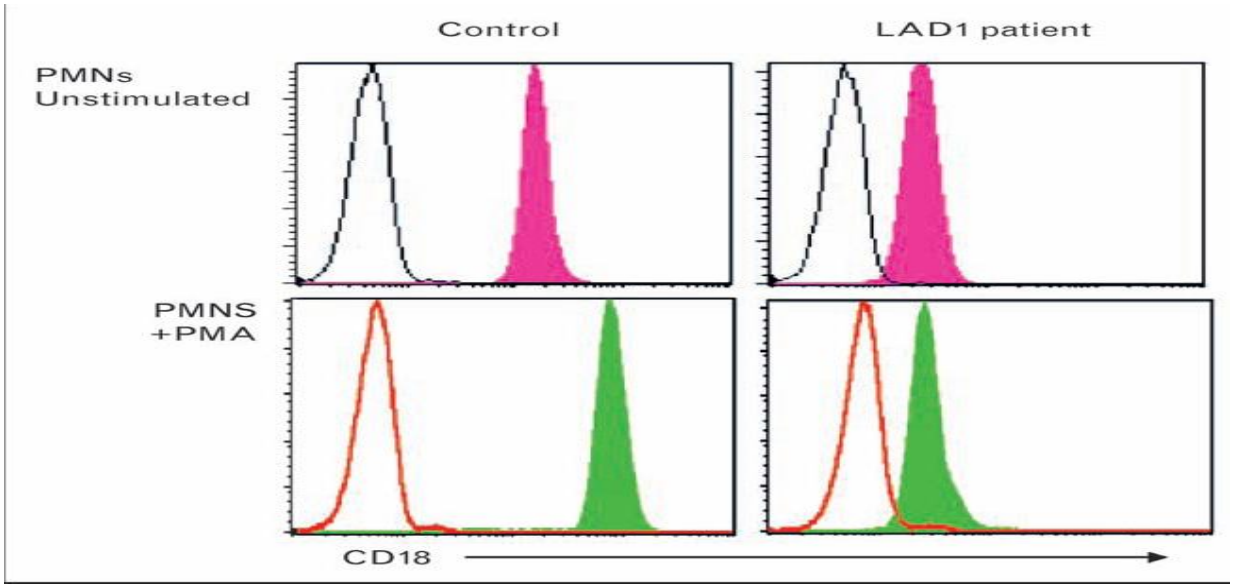
Defectos en la movilidad: deficiencias de *moléculas de adhesión (LAD)*

Infecciones recurrentes sin formación de pus – caida tardía de cordon- omfalitis

LAD tipo I β2 integrina (CD18)

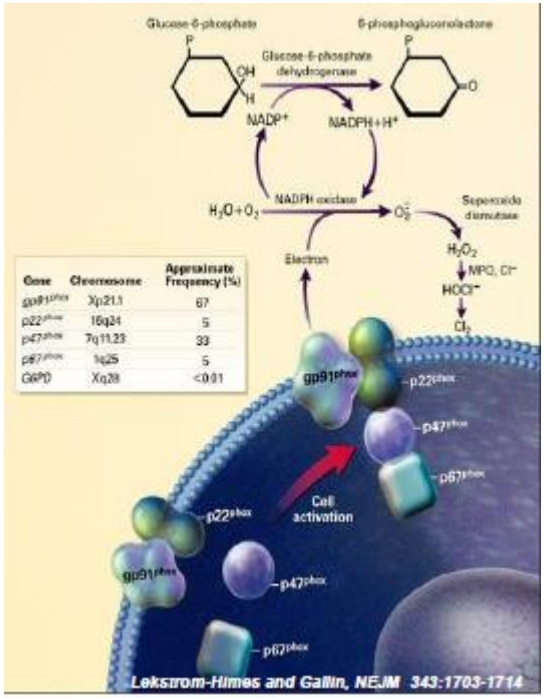
LAD tipo II Syalin Lewis X (CD15s)

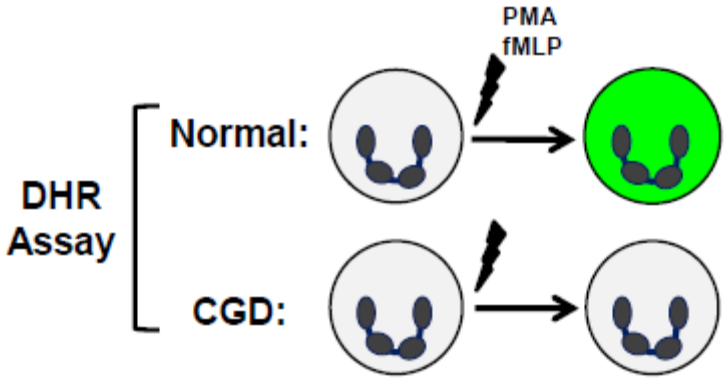
Expresión de moléculas de adhesión. CD18/CD11b



Enfermedad granulomatosa crónica

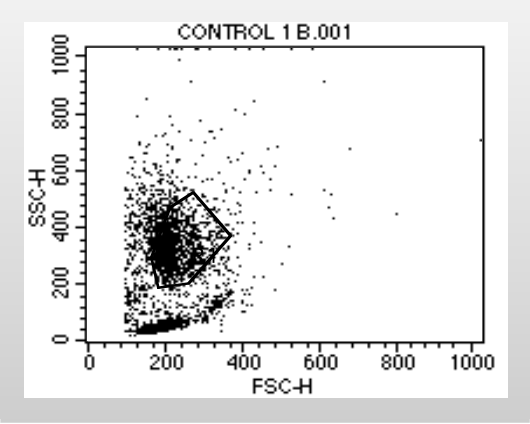
- ✓ **Enfermedad genéticamente heterogénea**
- ✓ **Infecciones recurrentes por bacterias y hongos e inflamación.**
- ✓**Defecto en la NADPH oxidasa responsable en los fagocitos del estallido respiratorio y la generación productos microbicidas.**

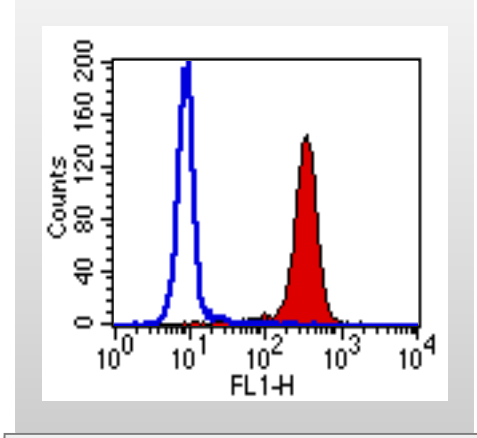




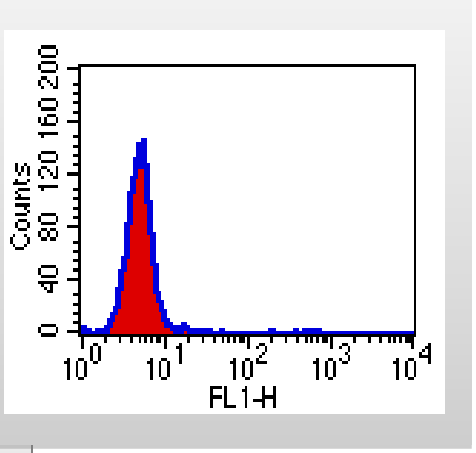
ENFERMEDAD GRANULOMATOSA CRONICA

TEST DHR

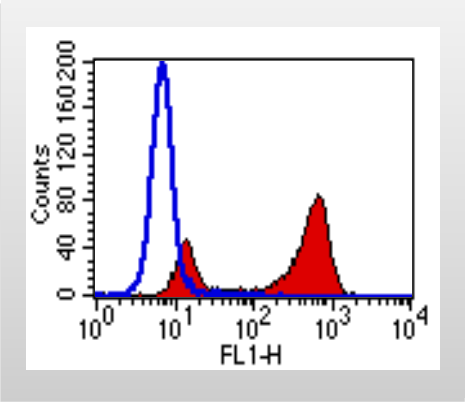




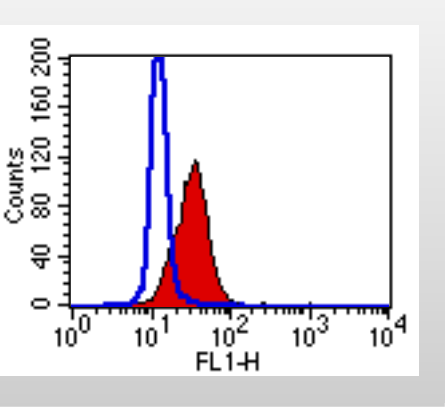
CONTROL NORMAL



EGC X

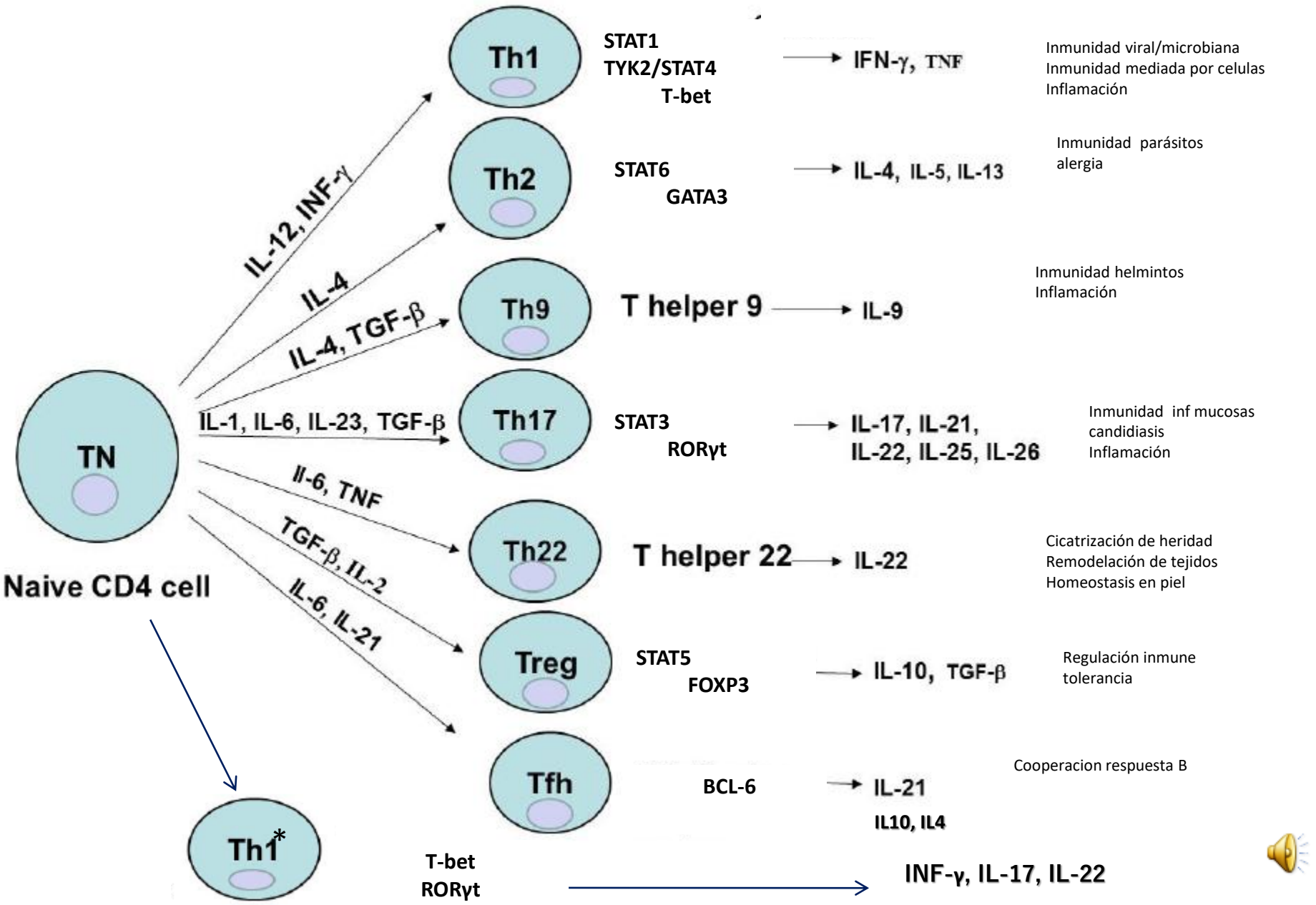


PORTADORA

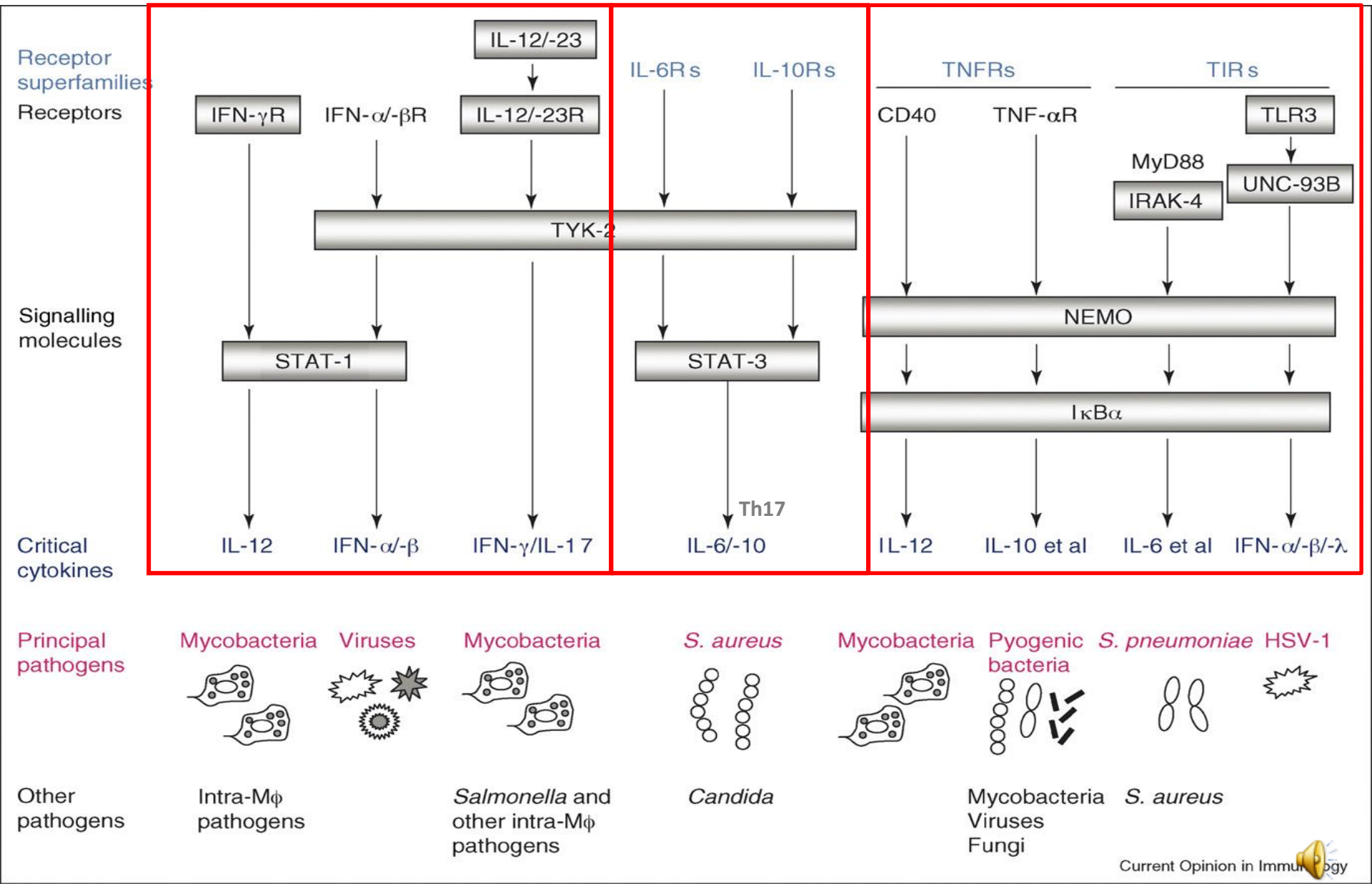


EGC AR

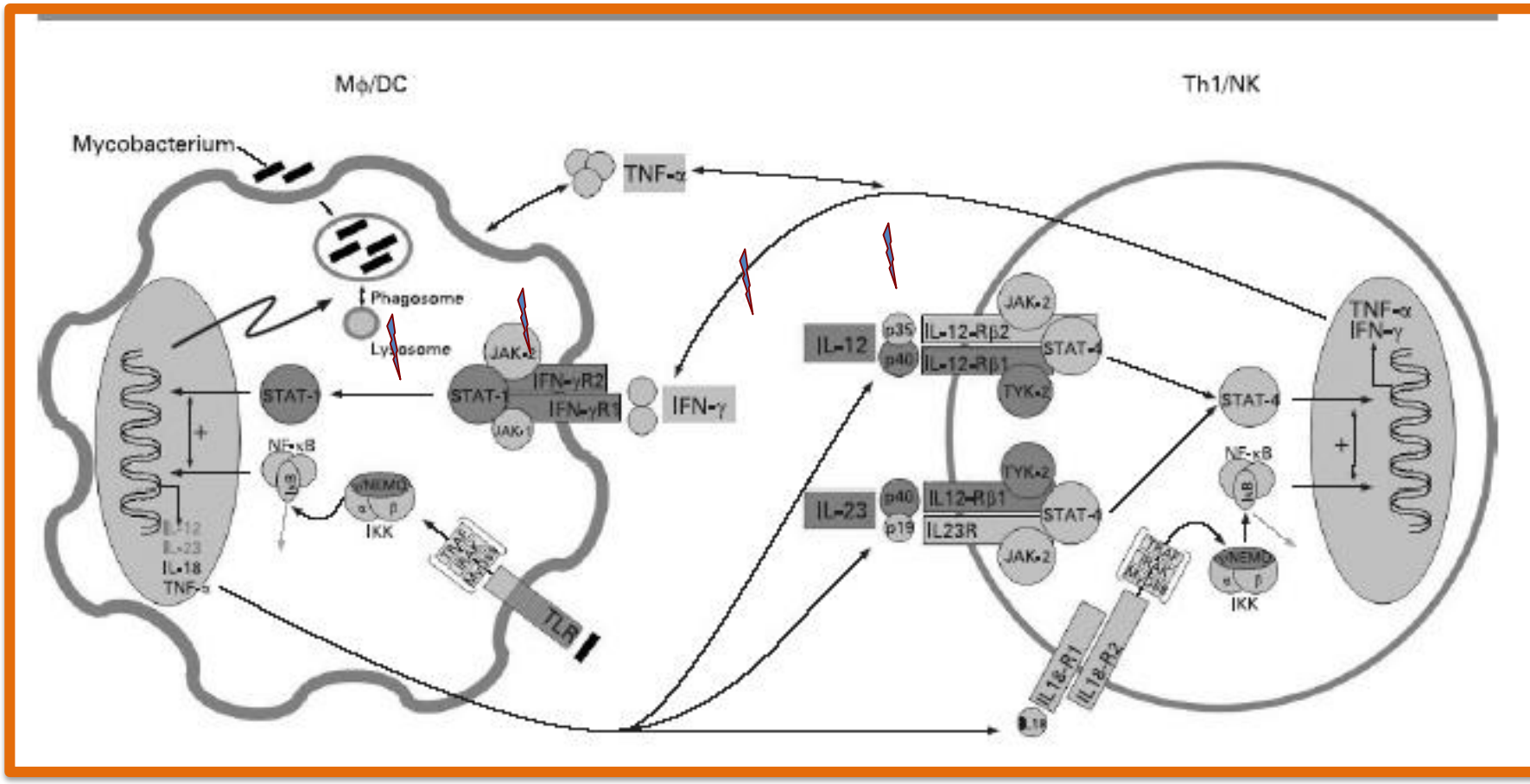
Subpoblaciones TCD4 memoria



Defectos que involucran la interfase entre inmunidad adaptativa e innata



Suceptibilidad mendeliana a micobacterias (MSMD) via INFγ/IL12-IL23

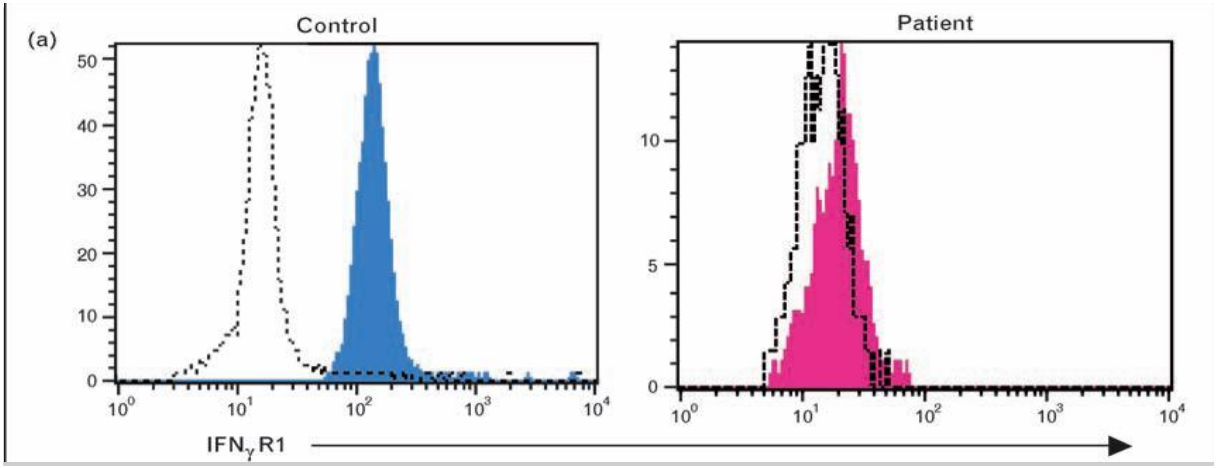


Expresión de receptores específicos
IL-12R β1(CD212)
IFNg R1 (CD119)

- Expresión de receptor de R1 INFγ (CD119) en monocitos

  Defectos parciales o completos con expresión de R

  Defectos completos con ausencia de R



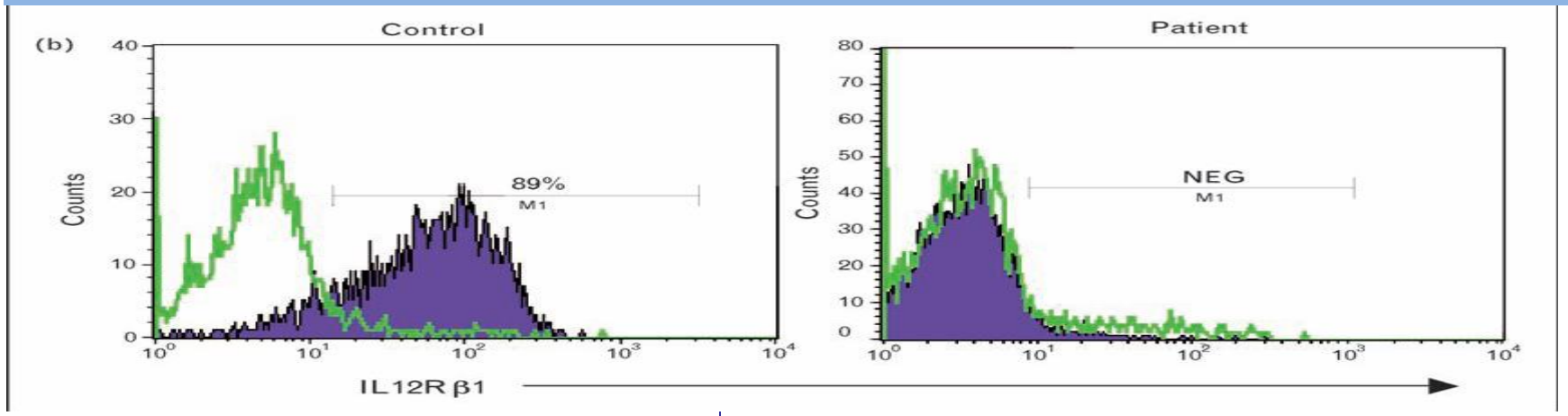
El 50% de las mutaciones en R1INF esta asociado a ausencia de expresión por CF. Pacientes con expresión de proteína no funcionante

Defectos parciales en formas AR o mutaciones AD asocian una incrementada expresión de receptor en la superfice celular

Evaluación eje IL12 / Rc IL12

Expresión de la Cadena β1 del RcIL12 (CD212)

	Normal (%)	Paciente (%)
basal	2	0
PHA+IL2	89	1



Defectos en la Vía INFγ/IL12

Expresión de receptores específicos

IL-12R (CD212)

IFNg R1 (CD119)

Estudios funcionales:Eventos tardíos

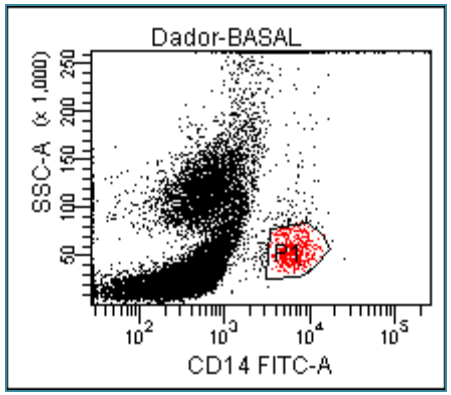
- **Estudio de los productos finales de cada vía (IFN-g, TNF-α, IL-12)**

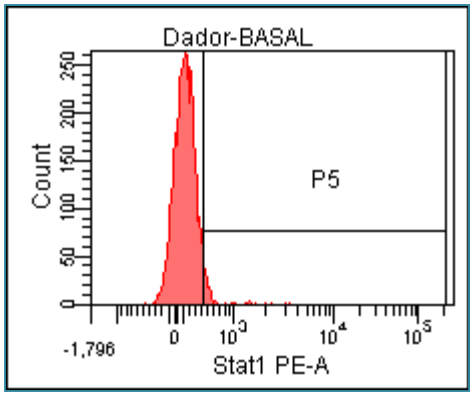
Estudios funcionales: Eventos tempranos

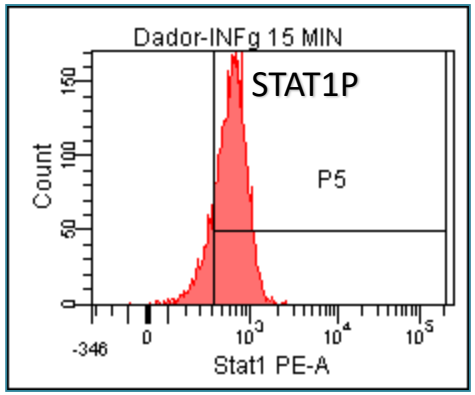
- **Fosforilación de Stat-1P**
- **Fosforilación de Stat-4P**

Fosforilación STAT1

- ESTIMULACION
- Fijación, permeabilización y marcación intracitoplasmática en monocitos.





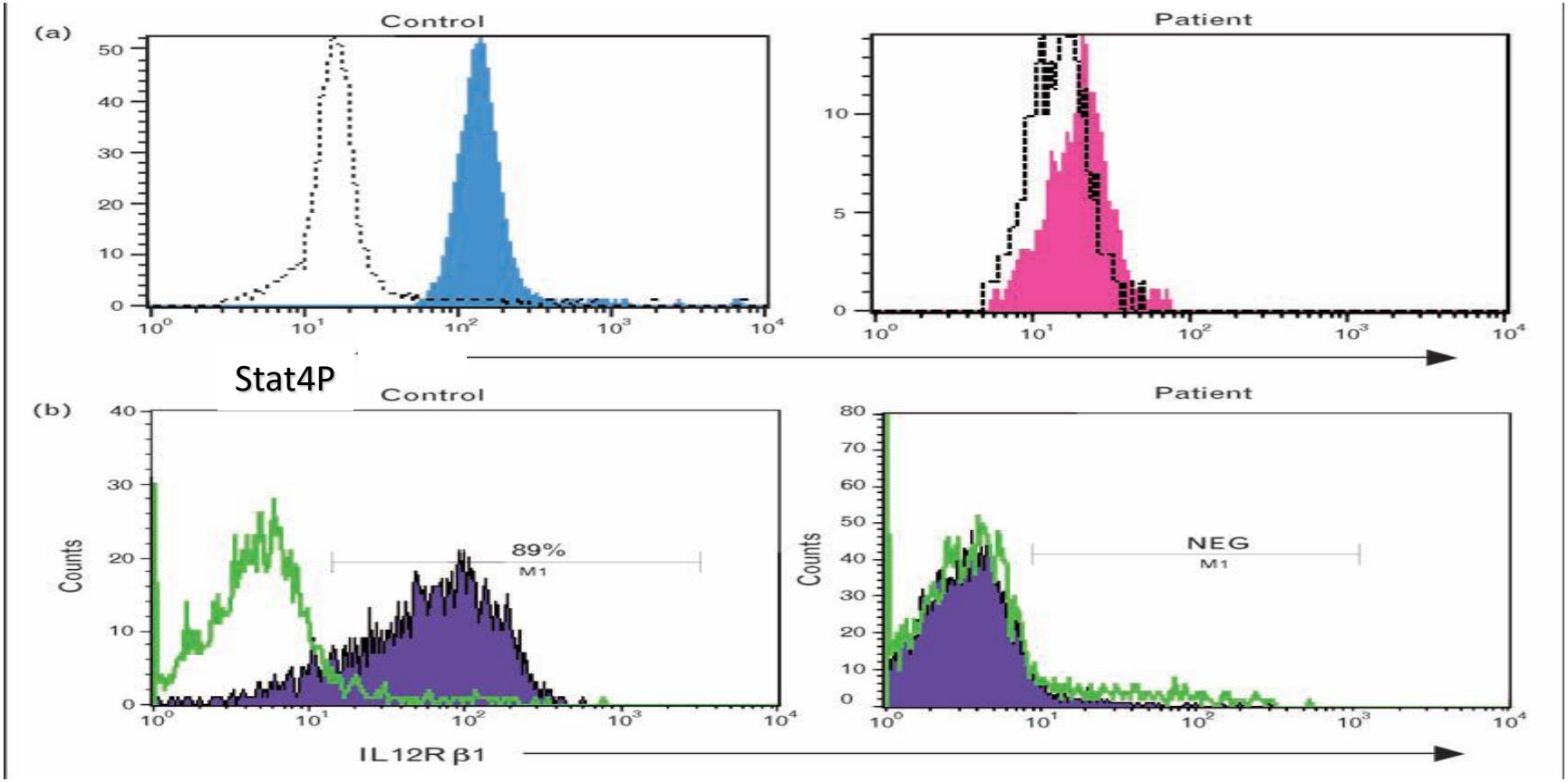


Basal (1/2 solo) 2% Estimulado con INF: 84%

Evaluación eje IL12 / Rc IL12

Eventos tempranos

Fosforilación de STAT4



Ensayo funcional para evaluar fosforilación de STAT4 en respuesta a IL12 e INFα en un paciente con mutación en IL12RB1.

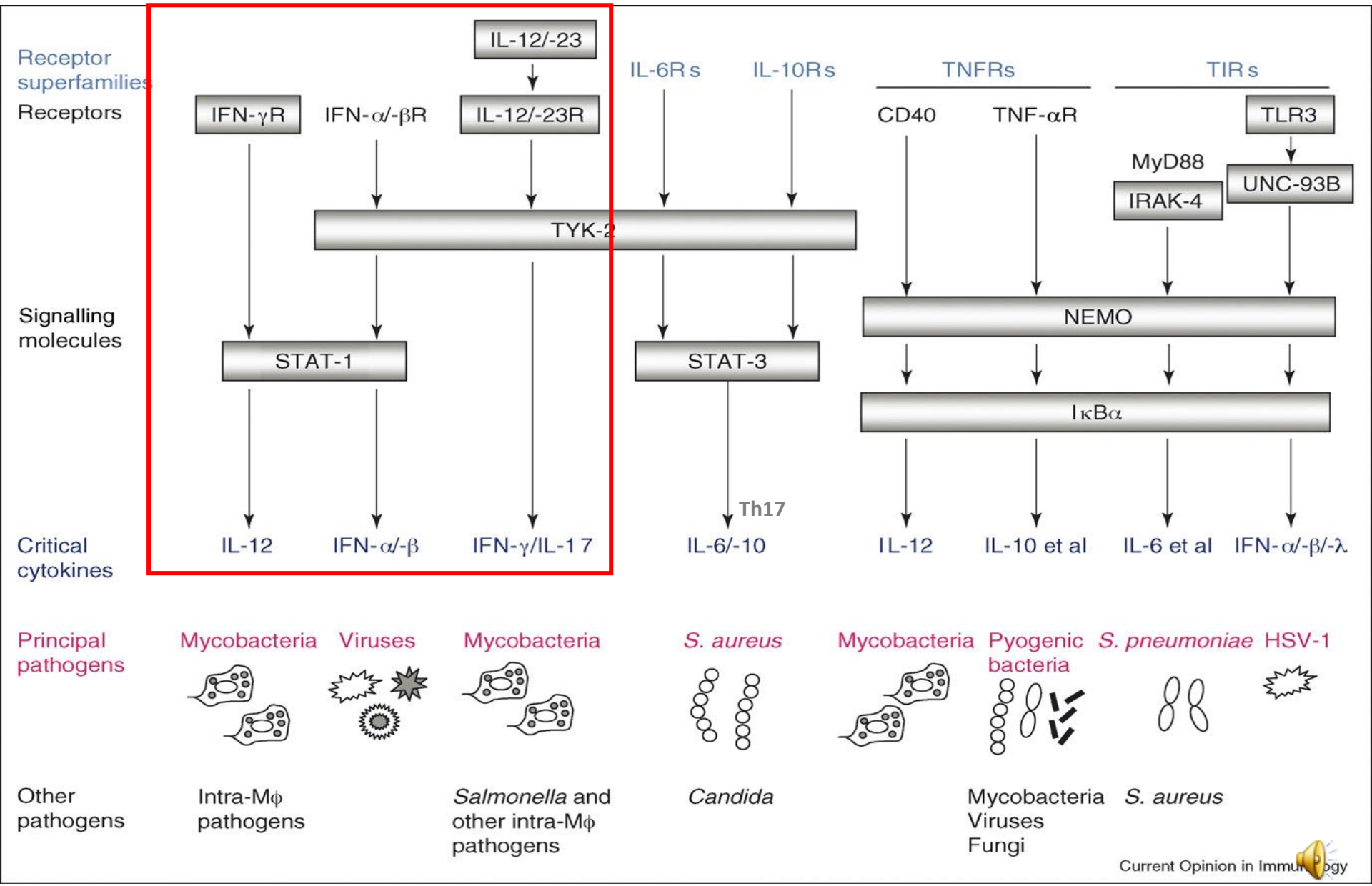
CASO #4

Paciente mujer 2a desarrolló BCGitis con osteomielitis en su brazo izquierdo.
Comenzó con hipotonía , pérdida de patrones de maduración y meningitis crónica. M. bovis se encontró en cultivos de LCR
Recibió tto con tuberculostáticos manteniendose los cultivos (+)
4 a: sepsis por S. *pneumoniae, candidiasis cutánea y micosis orofaríngea*

Laboratorio

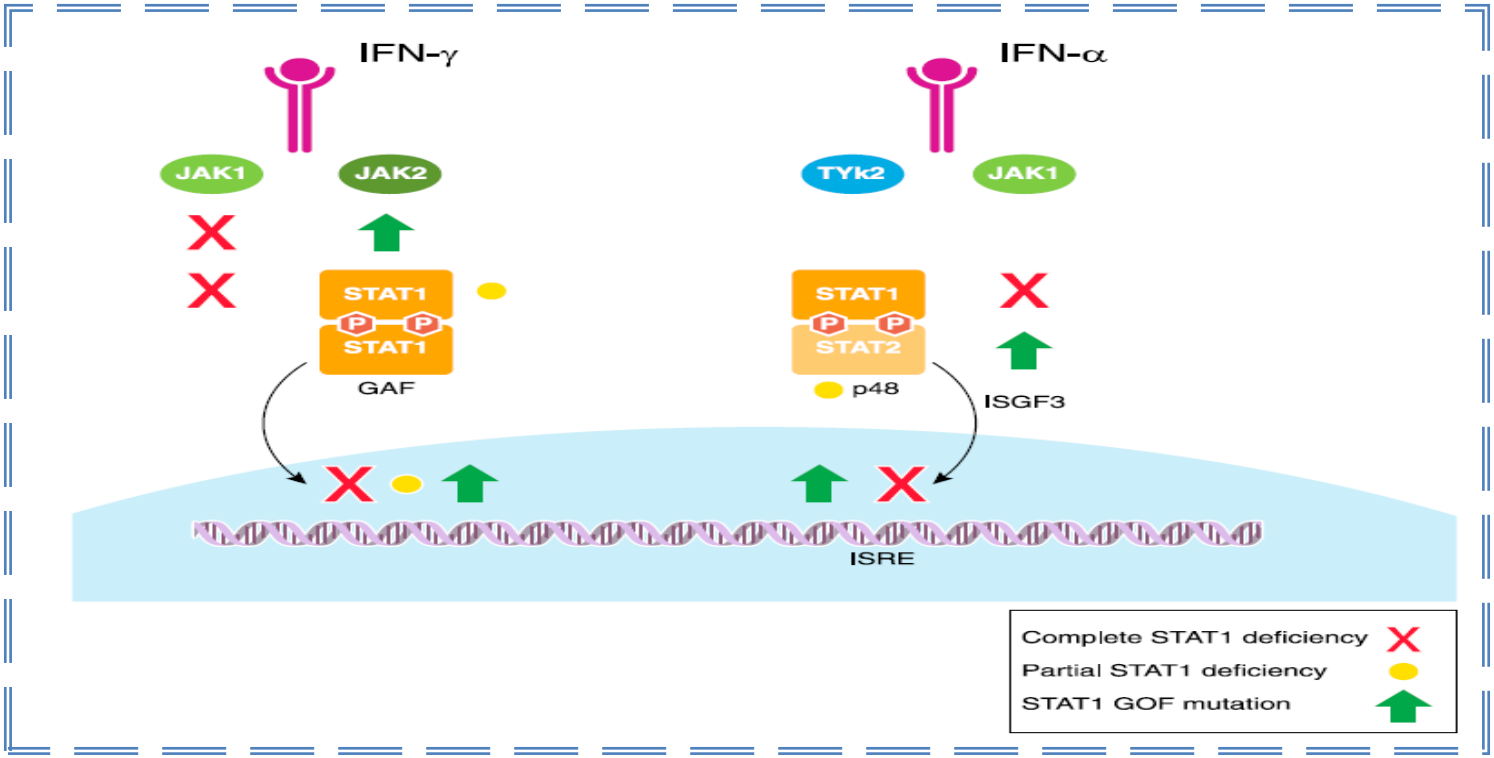
1. HIV negative.
2. leve hipogamaglobulinemia.
3. Respuesta proteica normal con alterada respuesta a polisacaridos
4. Subpoblaciones linfocitarias nomales con adecuada respuesta proliferativa
5. DHR normal

Defectos que involucran la interfase entre inmunidad adaptativa e innata

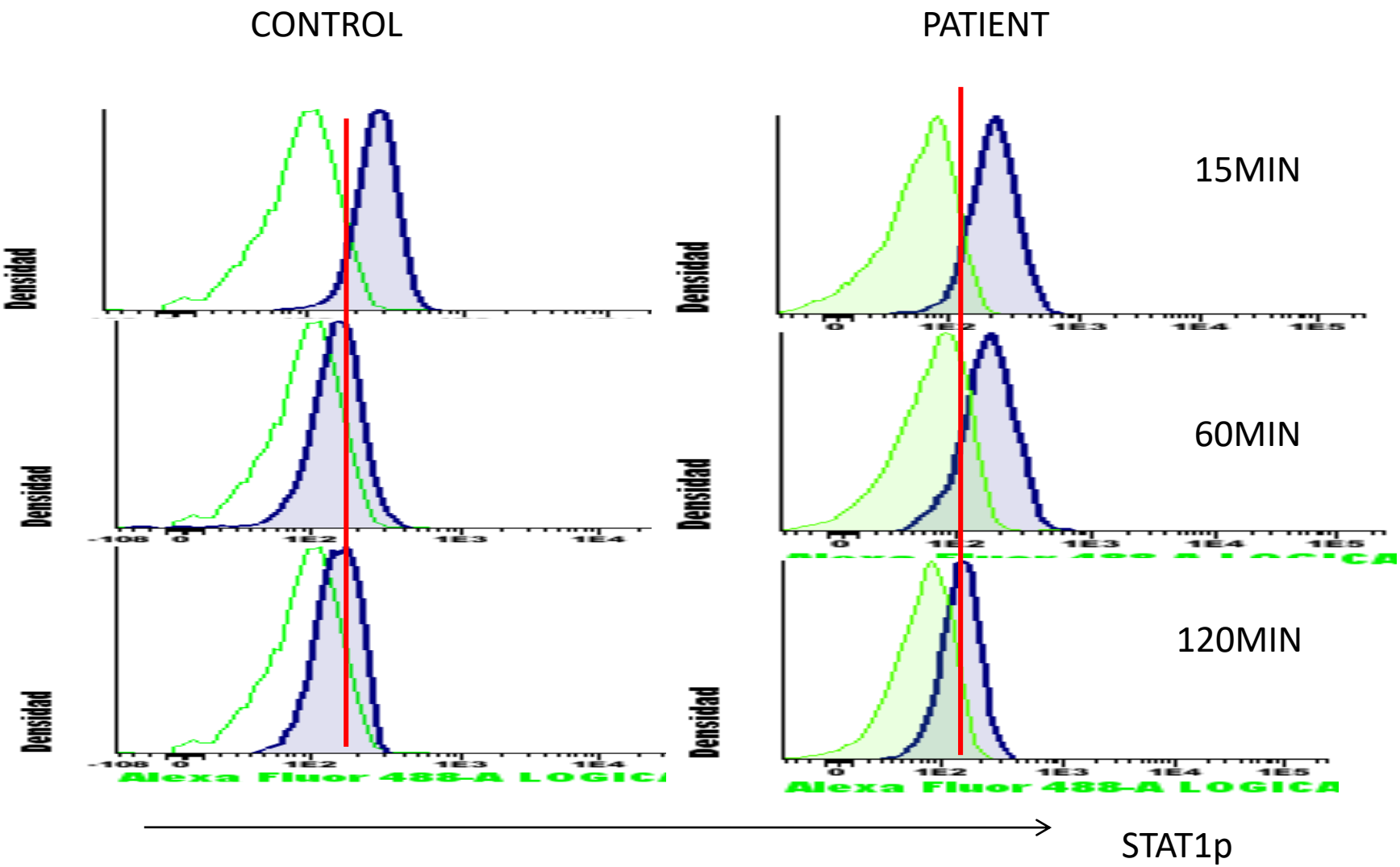


INFg-IL12 Via

1. Normal expresión de IL-12Rβ1(CD212) e IFN-gR1 (CD119)
2. Leve reducción de IL-12 en respuesta a hrIFN-g



Cinética de fosforilación de STAT1



heterozygous p.Q167H Stat-1 mutation

STAT1 GOF

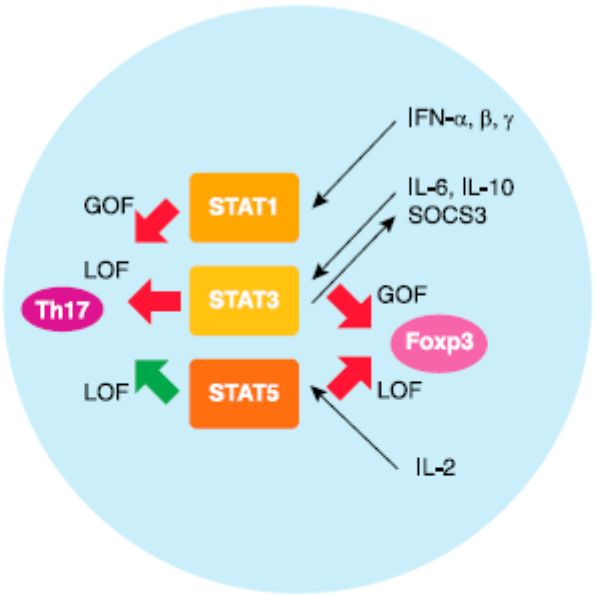
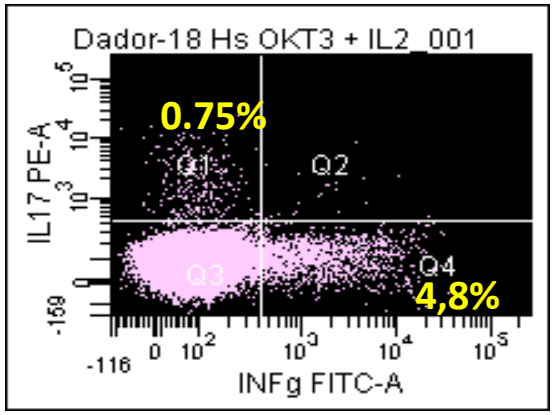
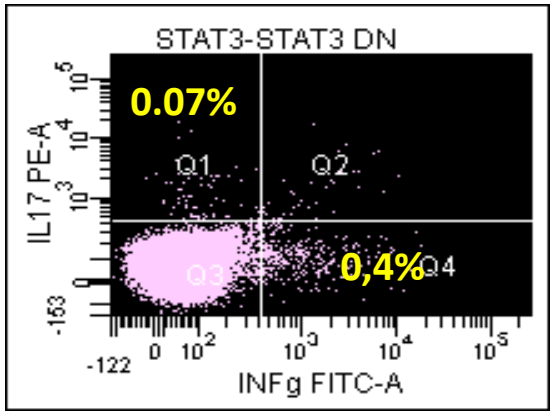


Figure 5. Interactions among STAT proteins. STAT3 GOF mutations are associated with decreased STAT1 and STAT5b phosphorylation, whereas STAT5b deficiency is characterized by enhanced STAT1 and STAT3 phosphorylation. STAT3 LOF and STAT1 GOF mutations reduce Th17 cell formation, whereas STAT5b deficiency is associated with elevated Th17 cells. Both STAT3 and STAT5b GOF mutations impair T_{reg} homeostasis. STAT1 GOF mutations are associated with reduced STAT3-dependent gene transcription.

CD4 act con aCD3

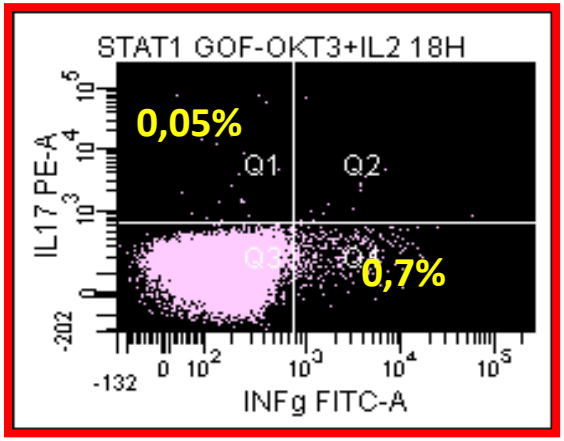


Control



STAT3DN

STAT1 GOF



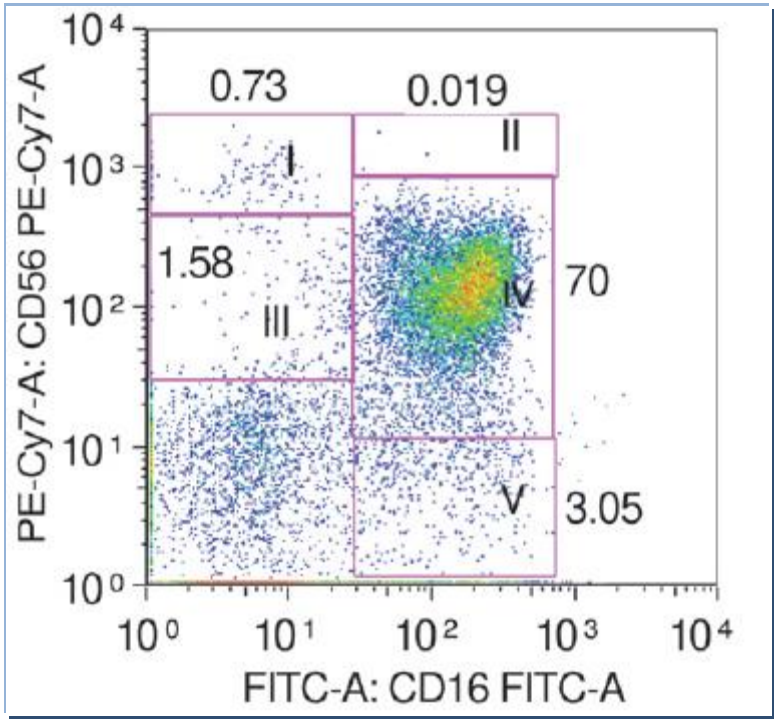
Sospecha de defectos de células NK y Linfocitos T citotóxicos

1

- Cuantificación
- Caracterización fenotípica

2

- Ensayos funcionales :
- CITOTOXICIDAD
- DEGRANULACIÓN



Enfermedades por Disregulación Inmune

IV. Diseases of immune dysregulation.
a : Hemophagocytic Lymphohistiocytosis HLH & EBV susceptibility

Hemophagocytic Lymphohistiocytosis (HLH)

Hypopigmentation:
Partial albinism . Decreased NK and CTL activities(cytotoxicity and/or degranulation). Bc and Tc: Nl

Chediak Higashi sd. ***LYST***
Recurrent infections, fever, (H)SM, bleeding tendency, progressive neurological dysfunction. Giant lysosomes (WBC), neutropenia, cytopenias, Specific hair shaft anomaly.

Griscelli sd type 2. ***RAB27a.***
Fever, (H)SM, cytopenias; Specific hair shaft anomaly.

Hermansky Pudlak sd type 2. ***AP3B1.***
Recurrent infections, pulmonary fibrosis, increased bleeding, neutropenia; Specific hair shaft anomaly.

Hermansky-Pudlak sd, type 10. ***AP3D1.***
Oculocutaneous albinism, severe neutropenia, recurrent infections, seizures, hearing loss and neurodevelopmental delay .

Familial Hemophagocytic Lymphohistiocytosis Syndromes: Fever, (H)SM, cytopenias,
Nl Bc. Increased activated Tc.
Decreased to absent NK and CTL activities cytotoxicity.

Perforin deficiency (FHL2). ***PRF1.***

UNC13D / Munc13-4 deficiency (FHL3). ***UNC13D.***

Syntaxin 11 deficiency (FHL4). ***STX11.***

STXBP2 / Munc18-2 deficiency (FHL5) ***STXBP2.***
Enteropathy

Susceptibility to EBV

RASGRP1 deficiency. ***RASGRP1.***
Recurrent pneumonia, herpes virus infections, EBV associated lymphoma. Increased IgA. Bc and Tc: Poor activation, proliferation, motility

CD70 deficiency. ***CD70*** *(TNFSF7).*
Hodgkin's lymphoma. Reduced IgM, IgG, IgA (75%) and reduced Ag-specific Ab responses (50%). Bc:poor antibody and memory responses. Tc:low Treg, poor activation and function

CTPS1 deficiency. ***CTPS1.***
Recurrent/chronic bacterial and viral infections (EBV, VZV), EBV lympho-proliferation, Bc non-Hodgkin lymphoma. Tc: poor proliferation to Ag

RLTPR (CARMIL2) deficiency. **RLTPR.** Recurrent bacterial, fungal and mycobacterial infections, viral warts, molluscum and EBV lymphoproliferative and other malignancy, atopy. Ig Nl to low, poor T dependent antibody response. Nl Bc. Tc: low Treg, high CD4, poor function.

ITK deficiency. ***ITK*** . EBV associated Bc lymphoproliferation, lymphoma, Nl or low IgG. Tc: Progressive decrease

MAGT1 deficiency (XMEN). ***MAGT1.XL*** EBV infection, lymphoma, viral infections, respiratory and GI infections. Low CD4 Low recent thymic emigrant cells, poor proliferation to CD3

PRKCD deficiency. ***PRKCD.*** Recurrent infections, EBV chronic infection, lymphoproliferation, SLE-like autoimmunity (nephrotic and antiphospholipid Sd). Low IgG. Low memory Bc high CD5 Bc

EBV associated HLH

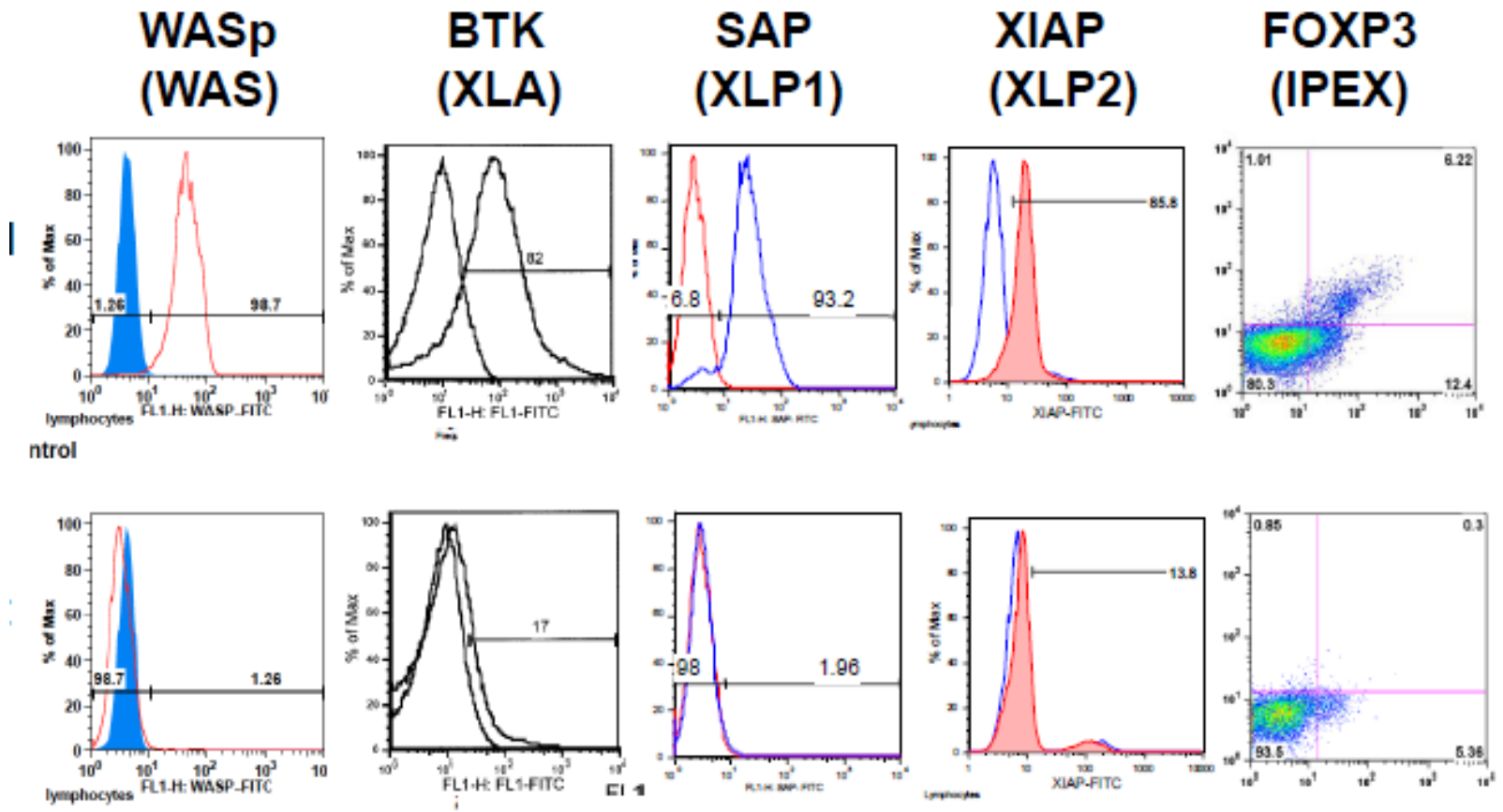
XL, XLP1. ***SH2DIA.***
Clinical and immunologic features triggered by EBV infection: lymphoproliferation, Lymphoma. Hypogamma globulinemia, Absent iNKT cells. Impaired NK cell and CTL cytotoxic activity . Reduced Memory B cells . SAP deficiency (CMF).

XL, XLP2. ***XIAP.***
Splenomegaly, lymphoproliferation, Colitis, IBD, hepatitis. Hypogamma-globulinemia, Low iNKT cells. Increased T cells susceptibility to apoptosis to CD95 and enhanced activation-induced cell death (AICD). Normal NK and CTL cytotoxic activity. XIAP def (CMF)

AR, CD27 deficiency . ***CD27*** *(TNFRSF7).*
Features triggered by EBV infection, aplastic anemia, low iNKTc lymphoma. Low Ig

FAAP24 deficiency. ***FAAP24.***
EBV-driven lymphoproliferative disease. Failure to kill autologous EBV transformed Bc.

Expresión de proteínas intracelulares en IDP



NKT (Va24/Vb11)

Enfermedades por Disregulación Inmune

IV. Diseases of immune dysregulation.
a : Hemophagocytic Lymphohistiocytosis HLH & EBV susceptibility

Hemophagocytic Lymphohistiocytosis (HLH)

Hypopigmentation:
Partial albinism . Decreased NK and CTL activities(cytotoxicity and/or degranulation). Bc and Tc: Nl

Chediak Higashi sd. *LYST*
Recurrent infections, fever, (H)SM, bleeding tendency, progressive neurological dysfunction. Giant lysosomes (WBC), neutropenia, cytopenias, Specific hair shaft anomaly.

Griscelli sd type 2. *RAB27a.*
Fever, (H)SM, cytopenias; Specific hair shaft anomaly.

Hermansky Pudlak sd type 2. *AP3B1.*
Recurrent infections, pulmonary fibrosis, increased bleeding, neutropenia; Specific hair shaft anomaly.

Hermansky-Pudlak sd, type 10. *AP3D1.*
Oculocutaneous albinism, severe neutropenia, recurrent infections, seizures, hearing loss and neurodevelopmental delay .

Familial Hemophagocytic Lymphohistiocytosis Syndromes: Fever, (H)SM, cytopenias,
Nl Bc. Increased activated Tc.
Decreased to absent NK and CTL activities cytotoxicity.

Perforin deficiency (FHL2). *PRF1.*

UNC13D / Munc13-4 deficiency (FHL3). *UNC13D.*

Syntaxin 11 deficiency (FHL4). *STX11.*

STXBP2 / Munc18-2 deficiency (FHL5) *STXBP2.*
Enteropathy

Susceptibility to EBV

RASGRP1 deficiency. *RASGRP1.*
Recurrent pneumonia, herpes virus infections, EBV associated lymphoma. Increased IgA. Bc and Tc: Poor activation, proliferation, motility

CD70 deficiency. *CD70 (TNFSF7).*
Hodgkin's lymphoma. Reduced IgM, IgG, IgA (75%) and reduced Ag-specific Ab responses (50%). Bc:poor antibody and memory responses. Tc:low Treg, poor activation and function

CTPS1 deficiency. *CTPS1.*
Recurrent/chronic bacterial and viral infections (EBV, VZV), EBV lympho-proliferation, Bc non-Hodgkin lymphoma. Tc: poor proliferation to Ag

RLTPR (CARMIL2) deficiency. RLTPR. Recurrent bacterial, fungal and mycobacterial infections, viral warts, molluscum and EBV lymphoproliferative and other malignancy, atopy. Ig Nl to low, poor T dependent antibody response. Nl Bc. Tc: low Treg, high CD4, poor function.

ITK deficiency. *ITK* . EBV associated Bc lymphoproliferation, lymphoma, Nl or low IgG. Tc: Progressive decrease

MAGT1 deficiency (XMEN). *MAGT1.XL* EBV infection, lymphoma, viral infections, respiratory and GI infections. Low CD4 Low recent thymic emigrant cells, poor proliferation to CD3

PRKCD deficiency. *PRKCD.* Recurrent infections, EBV chronic infection, lymphoproliferation, SLE-like autoimmunity (nephrotic and antiphospholipid Sd). Low IgG. Low memory Bc high CD5 Bc

EBV associated HLH

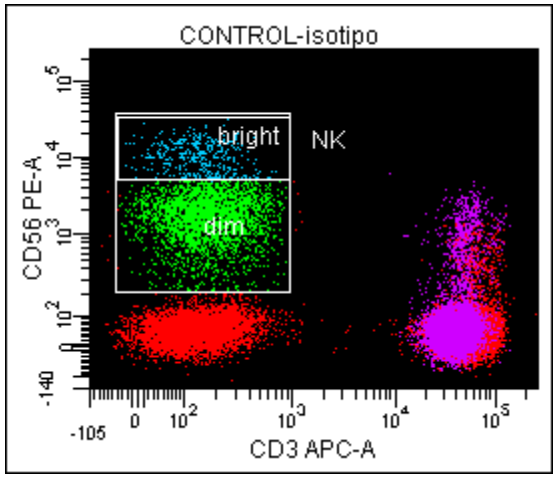
XL, **XLP1. *SH2DIA.***
Clinical and immunologic features triggered by EBV infection: lymphoproliferation, Lymphoma. Hypogamma globulinemia, Absent iNKT cells. Impaired NK cell and CTL cytotoxic activity . Reduced Memory B cells . SAP deficiency (CMF).

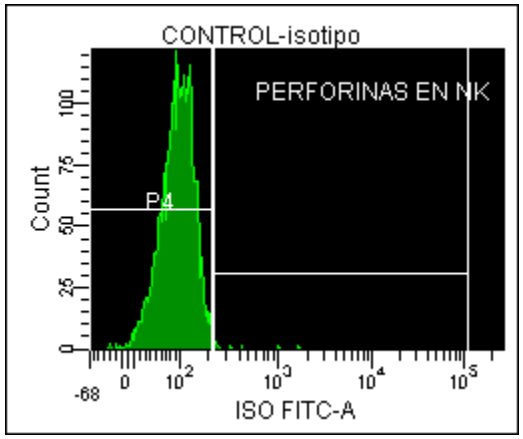
XL, **XLP2. *XIAP.***
Splenomegaly, lymphoproliferation, Colitis, IBD, hepatitis. Hypogamma-globulinemia, Low iNKT cells. Increased T cells susceptibility to apoptosis to CD95 and enhanced activation-induced cell death (AICD). Normal NK and CTL cytotoxic activity. XIAP def (CMF)

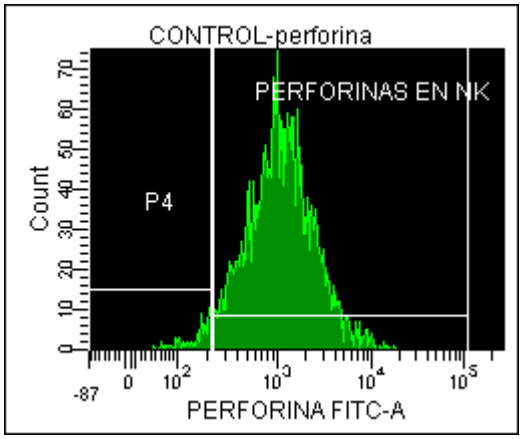
AR, **CD27 deficiency . *CD27 (TNFRSF7).***
Features triggered by EBV infection, aplastic anemia, low iNKTc lymphoma. Low Ig

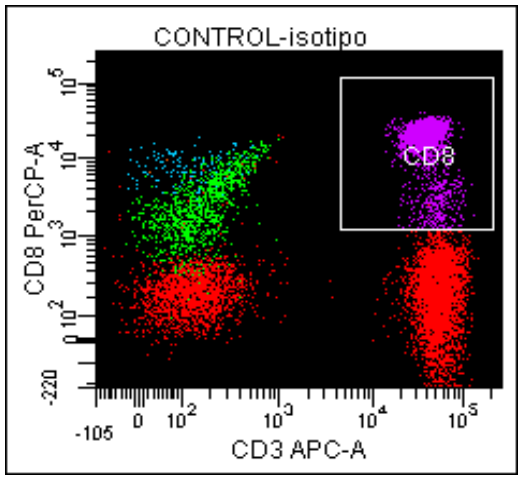
FAAP24 deficiency. *FAAP24.*
EBV-driven lymphoproliferative disease. Failure to kill autologous EBV transformed Bc.

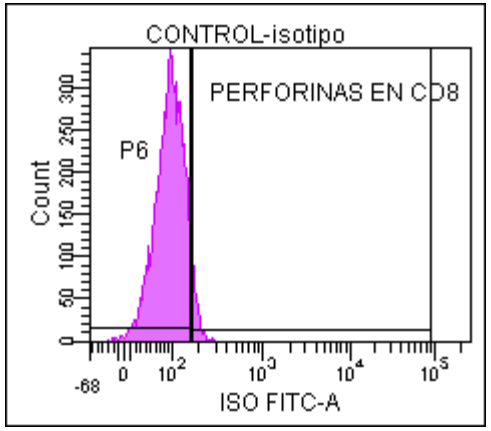
Expresión de Perforinas en células NK y LCD8

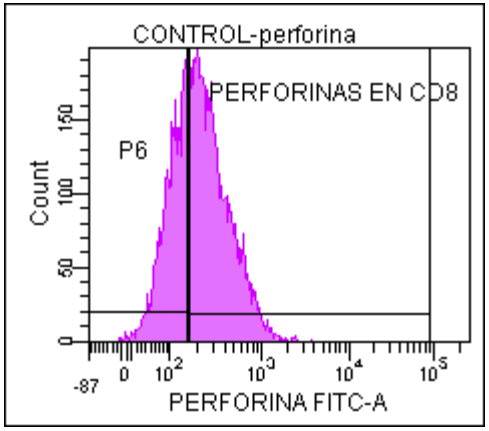




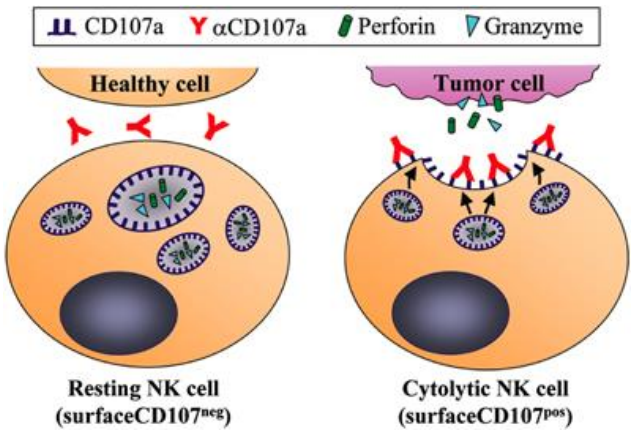




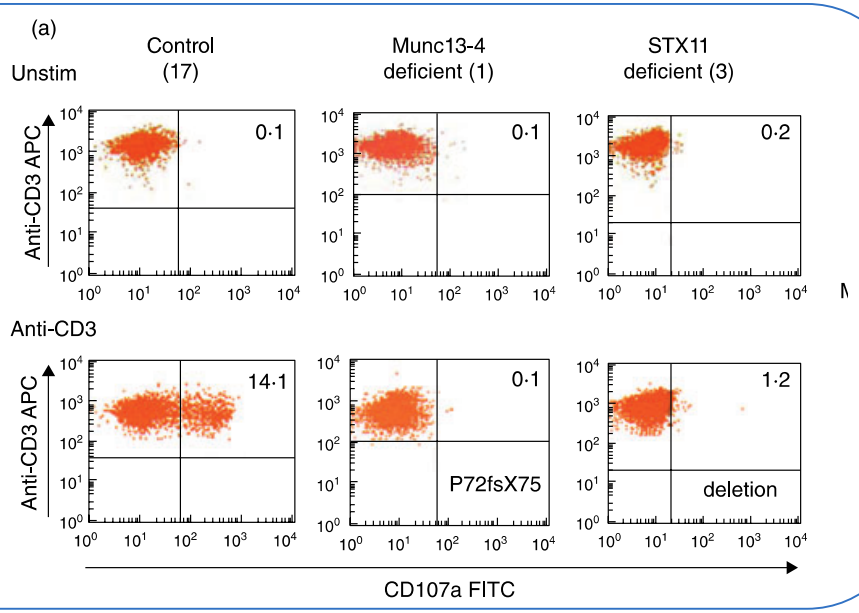




Ensayo de Degranulación: CD107a está presente en la membrana de los gránulos secretores de LT y NK. Se expresa en la superficie celular sólo cuando los gránulos secretores se fusionan con la membrana celular. Detección de CD107a en la superficie celular luego de estimulación implica una intacta exocitosis de los gránulos y función normal de la vía.

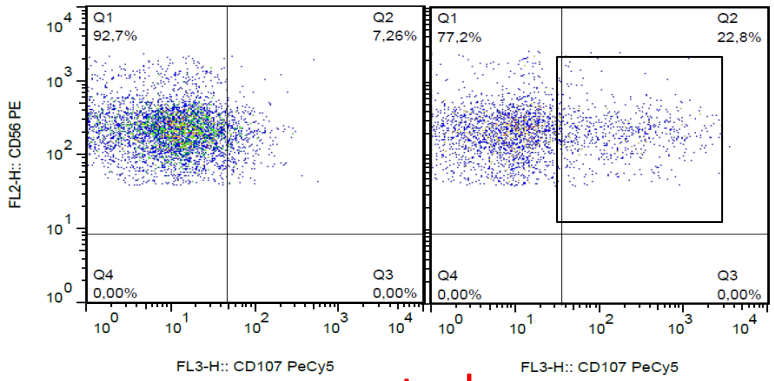


LTC



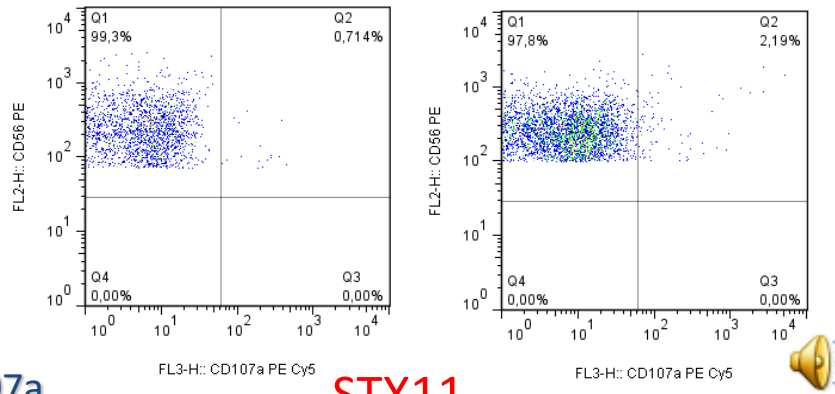
VR: $P_{2,5}$:10%- $P_{97,5}$: 30%

NK



control

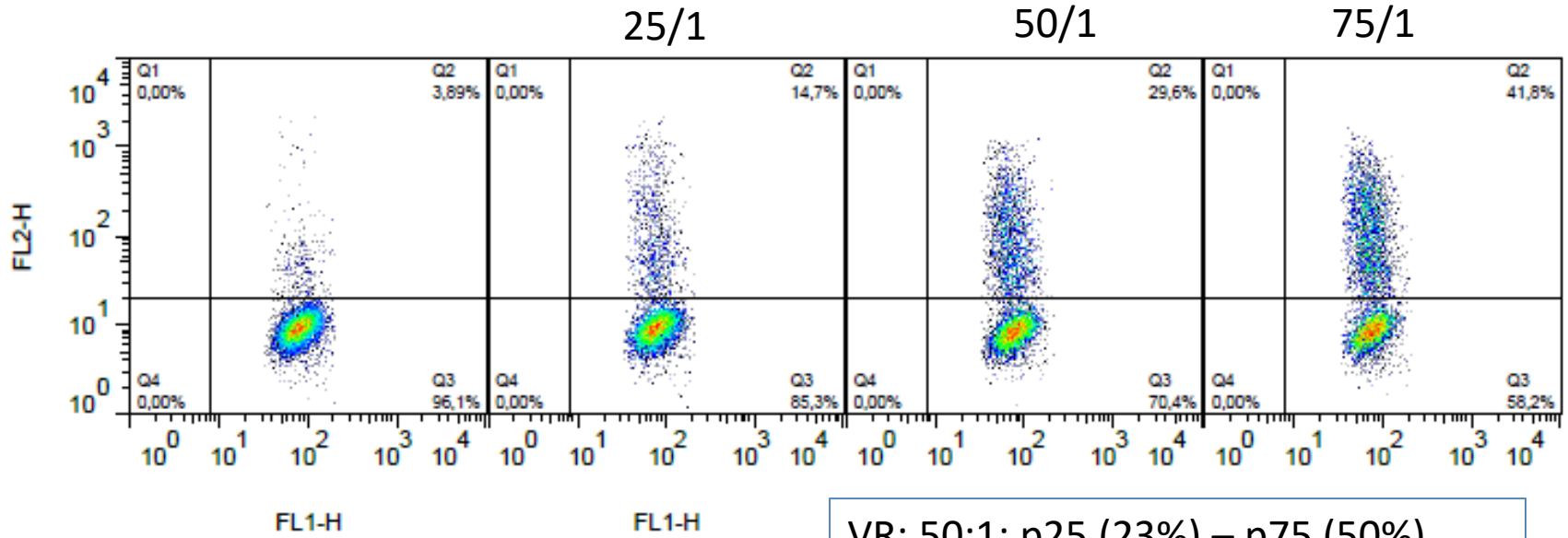
CD107a



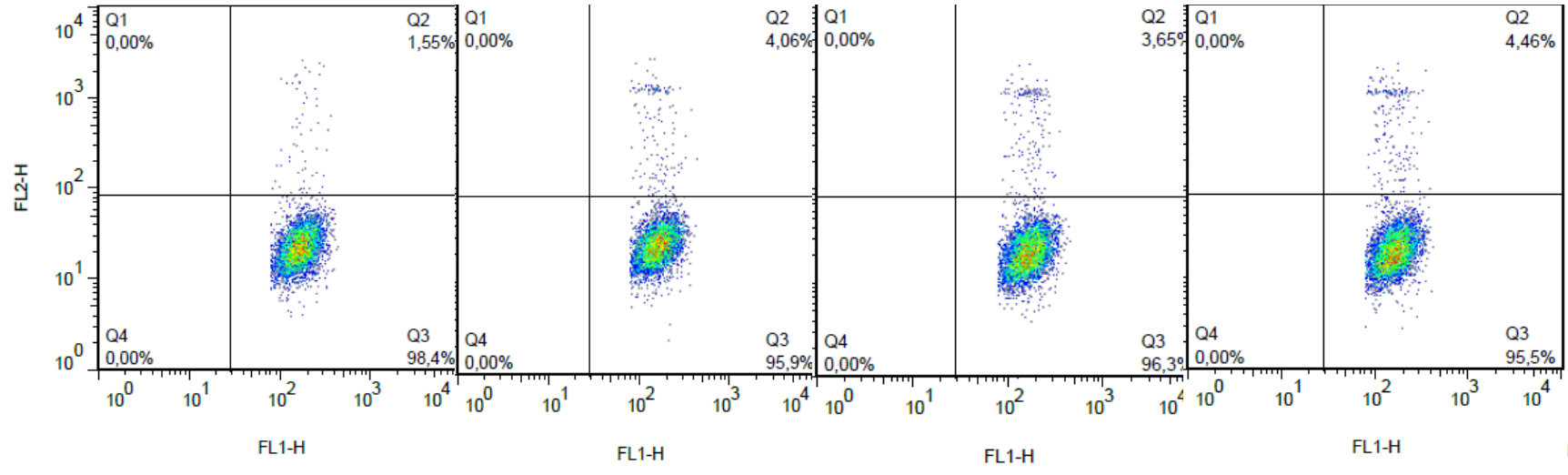
STX11

Ensayo de citotoxicidad de células NK por CF

Se enfrentan Células K562 (marcadas con CFSE)con distintas concentraciones de CMN y se evalúa la muerte celular por la incorporación de Ioduro de Propidio.



VR: 50:1: p25 (23%) – p75 (50%)



❑Todas estas metodologías son hoy herramientas fundamentales que correctamente estandarizadas e interpretadas contribuyen al diagnóstico de compromiso celular

❑ Apropiado y dirigido uso de los test de función inmune

DIAGNOSTICO MOLECULAR

MUCHAS GRACIAS

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